

# Congenital Fibrinogen Disorders: An Update

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## Abstract

Hereditary fibrinogen abnormalities comprise two classes of plasma fibrinogen defects: Type I, afibrinogenemia or hypofibrinogenemia, which has absent or low plasma fibrinogen antigen levels (quantitative fibrinogen deficiencies), and Type II, dysfibrinogenemia or hypodysfibrinogenemia, which shows normal or reduced antigen levels associated with disproportionately low functional activity (qualitative fibrinogen deficiencies). In afibrinogenemia and hypofibrinogenemia, most mutations of the *FGA*, *FGB*, or *FGG* fibrinogen encoding genes are null mutations. In some cases, missense or late truncating nonsense mutations allow synthesis of the corresponding fibrinogen chain but intracellular fibrinogen assembly and/or secretion are impaired. Afibrinogenemia is associated with mild-to-severe bleeding, whereas hypofibrinogenemia is most often asymptomatic. Thromboembolism may occur either spontaneously or in association with fibrinogen substitution therapy. Women with afibrinogenemia suffer from recurrent pregnancy loss but this can also occur in women with hypofibrinogenemia. Dysfibrinogenemia, caused mainly by missense mutations, is commonly associated with bleeding, thrombophilia, or both; however, most individuals are asymptomatic. Hypodysfibrinogenemia is a subcategory of this disorder. Even in specialized laboratories, the precise diagnosis of some fibrinogen disorders may be difficult. Determination of the molecular defects is important because it gives the possibility to confirm the diagnosis, to elaborate a diagnostic strategy, to distinguish in some cases that the patient is at risk of thrombosis rather than bleeding, and to enable prenatal diagnosis. However, genotype–phenotype correlations are not easy to establish. Replacement therapy is effective in treating bleeding episodes, but because the pharmacokinetics of fibrinogen after replacement therapy is highly variable among patients, it is important to adjust the treatment individually.

## Keywords

- ▶ bleeding disorder
- ▶ fibrinogen
- ▶ afibrinogenemia
- ▶ dysfibrinogenemia
- ▶ hypofibrinogenemia
- ▶ thrombosis

Diseases affecting fibrinogen may be acquired or inherited. Inherited disorders of fibrinogen are rare and can be subdivided into Type I and Type II disorders. Type I disorders (afibrinogenemia and hypofibrinogenemia) affect the quantity of fibrinogen in circulation (fibrinogen levels lower than 1.5 g/L). Type II disorders (dysfibrinogenemia and hypodysfibrinogenemia) affect the quality of circulating fibrinogen. While the first dysfibrinogenemia mutation was identified as early as 1968,<sup>1</sup> even before the genomic sequences of the

three fibrinogen genes were determined, the molecular basis of afibrinogenemia was elucidated much later.<sup>2</sup>

This review is an update of an article published in 2009 in this journal.<sup>3</sup> An important body of knowledge on congenital fibrinogen disorders was already available in 2009, thanks to international collaborative studies, national registries, as well as genetic studies. Most new data come from genotype analyses and the correlation of causative mutations with clinical phenotypes. This review will therefore discuss this

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aspect; however, the interested reader may like to consult two recent book chapters that cover extensively the mutations responsible for congenital fibrinogen disorders.<sup>4,5</sup> Before reviewing the various fibrinogen disorders, their causative mutations, diagnosis, and treatments, we will first introduce some aspects of fibrinogen synthesis and structure. Despite the progress made, many challenges remain, particularly to achieve more appropriate prevention and treatment of these diseases.

## Fibrinogen: Structure and Synthesis

The structure of fibrinogen, its synthesis, conversion to cross-linked fibrin, and its role in coagulation is well-described elsewhere,<sup>4,6</sup> but fibrinogen has other important physiological functions such as platelet cross-linking as part of primary hemostasis and a contribution to blood viscosity.

Fibrinogen is a 340 kDa glycoprotein synthesized in hepatocytes<sup>7</sup> that circulates in plasma at a concentration of 1.5 to 3.5 g/L. The core structure consists of two outer D regions<sup>8</sup> (or D domains) and a central E region (or E domain) connected through coiled, coil connectors (→Fig. 1). The molecule exhibits a twofold axis of symmetry perpendicular to the long axis, consisting of two sets of three polypeptide chains ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) that are joined in their amino terminal regions by disulfide bridges to form the E region. The outer D regions contain the globular C terminal domains of the  $\beta$  chain ( $\beta$ C) and  $\gamma$  chain ( $\gamma$ C). Unlike the  $\beta$ C and  $\gamma$ C domains, the C-terminal domains of the  $\alpha$  chain ( $\alpha$ C) are intrinsically unfolded and flexible and tend to be noncovalently tethered in the vicinity of the central E region.

The three genes encoding fibrinogen  $\beta$  (*FGB*),  $\alpha$  (*FGA*), and  $\gamma$  (*FGG*) are clustered in a region of ~50 kilobases on human chromosome 4.<sup>9</sup> *FGA* and *FGG* are transcribed from the reverse strand, in the opposite direction to *FGB*. Each gene is separately transcribed and translated to produce nascent polypeptides of 644 amino acids ( $\alpha$ ), 491 amino acids ( $\beta$ ), and 437 amino acids ( $\gamma$ ). Alternative splicing for *FGA* produces a minor extended isoform ( $\alpha$ -E), whereas alternative splicing of *FGG* produces the  $\gamma'$  isoform.<sup>10</sup>

During translocation of the single chains into the lumen of the endoplasmic reticulum (ER), a signal peptide is cotranslationally cleaved from each chain. Assembly proceeds in ER

with the formation of an  $\alpha$ - $\gamma$  or  $\beta$ - $\gamma$  intermediate. The addition of either a  $\beta$  or  $\alpha$  chain gives rise to an [ $\alpha$  $\beta$  $\gamma$ ] half-molecule, which dimerizes to form the functional hexamer.<sup>11</sup> The protein undergoes several posttranslational modifications in the Golgi complex.<sup>12</sup>

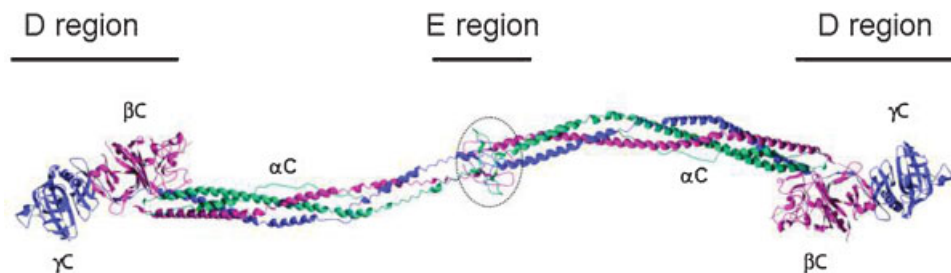
The mature molecule is constitutively secreted into the circulation, where it exhibits a half-life of ~4 days and a fractional catabolic rate of 25% per day.<sup>13</sup> In addition to plasma fibrinogen, blood contains an internalized intracellular fibrinogen pool that is stored within platelet  $\alpha$ -granules. Both megakaryocytes and platelets are capable of internalizing plasma fibrinogen via the fibrinogen glycoprotein IIb/IIIa ( $G_{PIIb-IIIa}$ ;  $\alpha_{IIb}\beta_3$ ) receptor.<sup>14</sup> Conversion of fibrinogen to a fibrin clot<sup>15,16</sup> occurs in three distinct phases: (1) enzymatic cleavage by thrombin to produce fibrin monomers; (2) self-assembly of fibrin units to form an organized polymeric structure; and (3) covalent cross-linking of fibrin by factor XIIIa.

Thrombin binds to its substrate, fibrinogen, through a fibrinogen recognition site in thrombin, referred to as exosite 1.<sup>17</sup> The fibrin clot itself also exhibits significant thrombin-binding potential. This nonsubstrate binding potential of fibrin for thrombin is referred to as antithrombin I.<sup>18</sup> Antithrombin I (fibrin) is an important inhibitor of thrombin generation that functions by sequestering thrombin in the forming fibrin clot and also by reducing the catalytic activity of fibrin-bound thrombin. Vascular thrombosis may result from absence of antithrombin I (as in afibrinogenemia, see below), reduced plasma  $\gamma'$  chain content,<sup>19</sup> or defective thrombin binding to fibrin as found in certain dysfibrinogenemias.

## Epidemiology and Clinical Features

### Afibrinogenemia

The disease was originally described in 1920<sup>20</sup> with an estimated prevalence of around 1 in 1,000,000 (→Table 1). According to the 2010 World Federation Hemophilia Annual Global Survey that included data from 106 countries, fibrinogen deficiencies account for 7% of cases of rare bleeding disorders and are more frequent in women than men.<sup>21</sup> In populations where consanguineous marriages are common, the prevalence of afibrinogenemia, as for other autosomal recessive coagulation disorders, is increased.<sup>22,23</sup>



**Fig. 1** Ribbon representation of native chicken fibrinogen.  $\alpha$  chains are in green,  $\beta$  chains are in purple, and  $\gamma$  chains are in blue. The globular C-terminal domains of the  $\beta$  and  $\gamma$  chains forming the D regions as well as the central E region that contains the N-terminal portions of all three chains are shown. Unlike the  $\beta$ C and  $\gamma$ C domains, the C-terminal domains of the  $\alpha$  chain ( $\alpha$ C) are flexible and tend to be noncovalently tethered in the vicinity of the central E region. (Modified from de Moerloose P, Neerman-Arbez M. Congenital fibrinogen disorders. *Semin Thromb Hemost* 2009;35(4):356–366).

**Table 1** Congenital fibrinogen deficiencies

	Afibrinogenemia	Hypofibrinogenemia	Dysfibrinogenemia
Prevalence	~1 in 1 million	More frequent <sup>a</sup>	More frequent <sup>a</sup>
Fibrinogen	Not detectable	~ 1.0 g/L or less	1.5–3.5 g/L
Symptoms	Bleeding, rare thrombosis	In general asymptomatic	Asymptomatic, bleeding and/or thrombosis
Treatment	Fibrinogen	Occasional fibrinogen	Fibrinogen and/or anticoagulants

<sup>a</sup>The prevalence of hypofibrinogenemia and dysfibrinogenemia is difficult to establish because of the large number of asymptomatic cases.

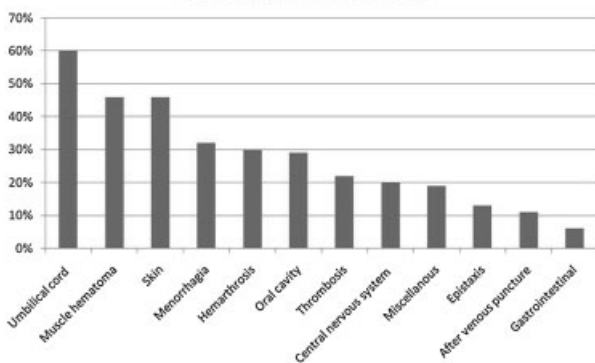
Several national registries<sup>22,24–26</sup> reported that bleeding due to afibrinogenemia usually manifests in the neonatal period with 85% of cases presenting umbilical cord bleeding<sup>22,27</sup> but a later age of onset is not unusual. Bleeding may occur in the skin, oral cavity, gastrointestinal tract, genitourinary tract, or the central nervous system with intracranial hemorrhage being the major cause of death. Prolonged bleeding after venous puncture has also been reported.<sup>28</sup> Joint bleeding which is common in patients with severe hemophilia is infrequent: In a series of 72 patients with severe fibrinogen deficiency, hemarthrosis was observed in 25% of cases.<sup>29</sup> Persistent damage to the musculoskeletal system and resulting handicap is also less frequent in patients with afibrinogenemia. There is an intriguing susceptibility of spontaneous rupture of the spleen in afibrinogenemic patients.<sup>27,30–32</sup> As described for factor XIII deficiency, quantitative abnormalities of fibrinogen can result in poor wound healing.<sup>33</sup> The clinical presentations of our registry of afibrinogenemic patients (*n* = 110) are shown in ►Fig. 2 (A. Casini, MD, unpublished data, 2013).

Afibrinogenemic women have an increased frequency of gynecologic and obstetric complications, such as menorrhagia, spontaneous recurrent abortions, antepartum, and postpartum hemorrhage.<sup>34,35</sup> Hemoperitoneum after rupture of the corpus luteum has also been observed.<sup>36</sup> Recently Levrat et al<sup>37</sup> reported that of the 13 members of a consanguineous Syrian family, 3 women suffered from iterative first-trimester miscarriages. The most frequent

bleeding symptoms were severe menorrhagia in all women homozygous for the causative mutation. The importance of fibrinogen in pregnancy has been demonstrated in studies with fibrinogen knockout mice where gestation cannot be maintained to term.<sup>38,39</sup>

As indicated in ►Fig. 2 and as described in most reports, paradoxically both arterial and venous thromboembolic complications are observed in afibrinogenemic patients.<sup>22,40–43</sup> These complications can occur in the presence of concomitant risk factors such as a co-inherited thrombophilic risk factor or after replacement therapy. However, in many patients, no known risk factors are present. Many hypotheses have been put forward to explain this predisposition to thrombosis. One explanation is that even in the absence of fibrinogen platelet aggregation is possible due to the action of von Willebrand factor<sup>44</sup> and, in contrast to hemophilic patients, afibrinogenemic patients are able to generate thrombin, both in the initial phase of limited production and also in the secondary burst of thrombin generation. In some patients, increase of prothrombin activation fragments or thrombin-antithrombin complexes has been observed, which may reflect an enhanced thrombin generation.<sup>45</sup> These abnormal levels can be normalized by fibrinogen infusions. Interestingly, an anti-thrombin role has also been attributed to fibrinogen, since in its absence, clearance of thrombin is impaired.<sup>46</sup> More importantly, as previously mentioned, fibrin also acts as anti-thrombin I by both sequestering and downregulating thrombin activity.<sup>47</sup> Thrombin, which is not trapped by the clot is available for platelet activation and smooth muscle cell migration and proliferation, particularly in the arterial vessel wall. In fibrinogen-deficient mice, thrombus formation is maintained but the thrombus is unstable and has a tendency to embolize.<sup>48</sup> Indeed, using an *in vivo* thrombosis model, Ni et al<sup>48</sup> analyzed thrombus growth in wild-type mice and in fibrinogen knockout mice. The number of embolized thrombi was sixfold higher in the Fg<sup>-/-</sup> mice than that in wild-type mice, with large emboli very often leading to vessel occlusion. Along this line, Remijn et al<sup>49</sup> have shown that absence of fibrinogen in human plasma results in large but loosely packed thrombi under flow conditions. Abnormal clot formation in the absence of fibrin may lead to an aberrant finding (e.g., isodense appearance of intraparenchymal bleeding on computed tomographic scans).

**Prevalence of symptoms at diagnosis of afibrinogenemia (n=110)**



**Fig. 2** Clinical presentation among 110 probands with afibrinogenemia. Miscellaneous: hematuria, postsurgery, retroperitoneal, and hemoptysis.

**Hypofibrinogenemia**

Hypofibrinogenemia was first reported in 1935.<sup>50</sup> Because hypofibrinogenemia (fibrinogen levels below 1.5 g/L) is often

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**Table 2** Mutations associated with liver disease

cDNA	Nascent chain (mature)	Type, Name
928G > C	G310R (G284R)	Hypofibrinogenemia, Brescia
1018C > A	T(340) (T314P)	Hypofibrinogenemia, Al du Pont
1116_1129 + 1del	del372–376 (del346–350)	Hypofibrinogenemia, Angers
1201C > T	R401W (R375W)	Hypofibrinogenemia, Aguadilla

Abbreviation: cDNA, complementary deoxyribonucleic acid.

caused by heterozygosity for a fibrinogen gene mutation, it is much more frequent than afibrinogenemia (► **Table 1**). Indeed, if one applies the Hardy Weinberg binomial distribution of alleles in the population to afibrinogenemia, carriers of fibrinogen deficiency causing mutations could be as frequent as 1 of 500. These patients are usually asymptomatic with fibrinogen levels around 1.0 g/L, levels that are high enough to protect against bleeding<sup>51</sup> and maintaining pregnancy.<sup>52</sup> However, they can bleed (as normal individuals) when exposed to trauma, or if they have a second associated hemostatic abnormality. Hypofibrinogenemic women may also suffer from pregnancy loss or postpartum hemorrhage. Indeed, in a family of hypofibrinogenemic patients, the only bleeding problem reported was postpartum hemorrhage, which occurred in all four female members.<sup>53</sup> Liver disease occurs in rare cases of hypofibrinogenemia (► **Table 2**). Here, the impaired release of the abnormal fibrinogen results in the accumulation of aggregates in the ER of hepatocytes.<sup>54–57</sup>

### Dysfibrinogenemia and Hypodysfibrinogenemia

Dysfibrinogenemias and hypodysfibrinogenemias are generally associated with autosomal dominant inheritance, caused by heterozygosity for missense mutations in the coding region of one of the three fibrinogen genes, and so they are more frequent than Type I disorders. Dysfibrinogenemia was first reported in 1958<sup>58</sup> and more than 500 cases have been reported to date.

Patients with inherited dysfibrinogenemia are frequently asymptomatic. Indeed, dysfibrinogenemia is usually discovered incidentally because of abnormal coagulation tests or because a case of dysfibrinogenemia has been previously discovered in the family. However, some patients suffer from bleeding, thromboembolic complications, or both (► **Table 1**). A compilation of more than 260 cases of dysfibrinogenemia revealed that 55% of the patients had no clinical complications, 25% exhibited bleeding, and 20% a

tendency to thrombosis, mainly venous.<sup>59</sup> Patients with dysfibrinogenemia associated with hemorrhage bleed most often after trauma, surgery, or in the postpartum. Two mechanisms may explain most of the cases of thrombosis associated with dysfibrinogenemia: (1) The abnormal fibrinogen is defective in binding thrombin, which results in elevated levels of thrombin. (2) The abnormal fibrinogen forms a fibrin clot that is resistant to plasmin degradation. Recently, a high prevalence of dysfibrinogenemia among patients with chronic thromboembolic pulmonary hypertension was highlighted.<sup>60</sup> Genetic analysis revealed that 5 of 33 patients were heterozygous for fibrinogen gene mutations. Functional analysis disclosed abnormalities in fibrin polymer structure and/or lysis in these five patients. These findings may also be relevant to patients with deep vein thrombosis or pulmonary embolism who have incomplete clot resolution.<sup>40</sup> Despite the possible occurrence of thrombosis in congenital fibrinogen disorders, when patients with deep vein thrombosis are screened for thrombophilia, the prevalence of dysfibrinogenemia is very low (0.8% based on a review of 2,376 patients), so systematic testing for dysfibrinogenemia in patients with thrombophilia is not recommended.<sup>61</sup>

Women with dysfibrinogenemia can also suffer from spontaneous abortions. The problems during and after pregnancy are not necessarily correlated to the fibrinogen concentration. Thrombosis may also occur in the postpartum.

Some mutations in the  $\alpha$ -chain of fibrinogen are associated with a particular form of hereditary amyloidosis (► **Table 3**).<sup>62</sup> The E545V (E526V) amino acid substitution is the most common of these mutations. The abnormal fibrinogen fragments form amyloid fibrils and the extracellular deposition of these fibrils leads to renal failure. Chronic renal dialysis is performed for managing renal failure. Renal transplantation should be considered as an alternative to chronic dialysis; however, renal transplantation is not a curative solution for hereditary renal amyloidosis-related renal

**Table 3** Mutations associated with renal amyloidosis

cDNA	Nascent chain (mature)	Type
1622delT	V541Afs (V522Afs)	Dysfibrinogenemia
1629delG	T544Lfs (T525Lfs)	Dysfibrinogenemia
1634A > T	E545V (E526V)	Dysfibrinogenemia
1718G > T	R573L (R554L)	Dysfibrinogenemia

Abbreviation: cDNA, complementary deoxyribonucleic acid.

failure, because continuous fibrinogen-related amyloid deposition ultimately results in allograft destruction. In a long-term follow-up of patients with hereditary fibrinogen A $\alpha$ -chain amyloidosis vascular disease was an important cause of morbidity and mortality.<sup>63</sup> Combined liver and kidney transplantation prevents further amyloid deposition in the renal allograft and elsewhere but is associated with additional perioperative and subsequent risks.<sup>64</sup>

Despite abundant biological and molecular information, clinical information on patients is usually poor with only short follow-up periods reported. Furthermore, the limited data of comprehensive family studies does not enable accurate calculation of the penetrance of a phenotype. In a series of 37 probands from 12 unrelated families with 5 different defects, more than half of probands experienced 1 or more undue bleeding episodes, easy bruising being the most common. Nine (19%) probands, all older than 50 years, had at least one episode of arterial or venous thrombosis.<sup>65</sup>

## Laboratory Assays

Initial screening tests for fibrinogen disorders should include fibrinogen concentration, measured functionally and immunochemically, thrombin time (TT), and reptilase (a snake venom that removes only FpA but also triggers fibrin polymerization) time, backed by genetic analyses (genotype analysis).

## Phenotype Analysis

Absence of immunoreactive fibrinogen is essential for the diagnosis of congenital afibrinogenemia. All coagulation tests that depend on the formation of fibrin as the end point, that is, prothrombin time (PT), activated partial thromboplastin time (aPTT), or TT are infinitely prolonged. Plasma activity of all other clotting factors is normal. Some abnormalities in platelet functions tests can be observed that can be reversed upon addition of fibrinogen.<sup>66</sup> Because fibrinogen is one of the main determinants of erythrocyte sedimentation, it is not surprising that afibrinogenemic patients have very low erythrocyte sedimentation rates. When skin testing is performed for delayed hypersensitivity, there is no induration due to the lack of fibrin deposition.<sup>67</sup>

*Hypofibrinogenemia* is defined as a proportional decrease of functional and immunoreactive fibrinogen. Coagulation tests depending on the formation of fibrin are variably prolonged, the most sensitive assay being the TT.

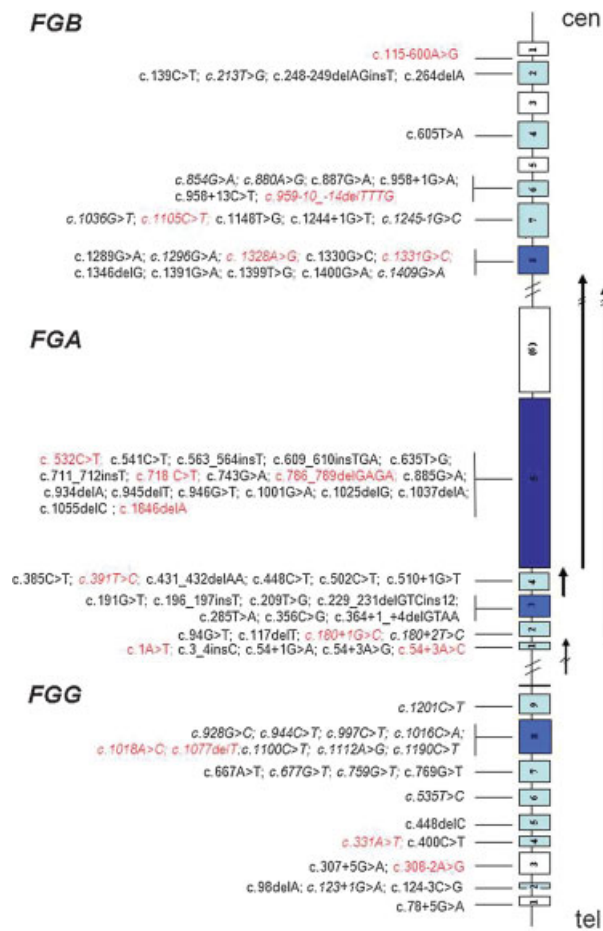
*Dysfibrinogenemia* is diagnosed by a discrepancy between clottable and immunoreactive fibrinogen. However, even in specialized laboratories, this diagnosis can be difficult because the sensitivity of the tests depends on the specific mutation, reagents, and techniques. A diagnostic algorithm based on the TT as an initial test has been proposed.<sup>68</sup> However, some dysfibrinogenemias (e.g., Oslo 1) have a normal TT. In 27 patients with dysfibrinogenemia, the PT-derived method overestimated the fibrinogen by approximately five times the value measured by the Clauss assay.<sup>69</sup> While fibrinogen measured by the PT-derived method correlated with fibrinogen antigen, concentrations measured by

the immunological fibrinogen or heat fibrinogen method, fibrinogen measured by the Clauss method correlated with functional coagulation parameters, such as reptilase time, TT or PT. In classical dysfibrinogenemias, the functional assay of fibrinogen yields low levels compared with the immunological assays but levels are sometimes concordant and the functional level may even be normal.

## Genotype Analysis

In 1999, the first causative mutation for afibrinogenemia, a large, recurrent deletion, was identified in the fibrinogen gene cluster.<sup>2</sup> Since this first report, more mutations, the majority in *FGA*, have been identified in patients with afibrinogenemia (in homozygosity or in compound heterozygosity) or in hypofibrinogenemia, since a large number of these patients are in fact asymptomatic heterozygous carriers of afibrinogenemia mutations (►Fig. 3). Causative mutations can be divided into two main classes: null mutations with no protein production at all and mutations producing abnormal protein chains that are retained inside the cell. Although many more mutations have been described in various formats, ►Fig. 3 shows only those published in peer-reviewed, international journals for which functional data have established the causative nature of the mutation, or those that are sufficiently severe (e.g., frameshift mutations, early truncating nonsense mutations) to leave no reasonable doubt as of their pathological implication. Null mutations, that is, large deletions, frameshift, early-truncating nonsense, or splice-site mutations, account for the majority of afibrinogenemia alleles, as expected, given the phenotype of the disorder, that is, complete deficiency of fibrinogen in circulation. Of particular interest therefore are missense mutations leading to complete fibrinogen deficiency. These are clustered in the highly conserved C-terminal globular domains of the B $\beta$  and  $\gamma$  chains. Functional studies of these mutations in transfected cells have demonstrated either impaired assembly or impaired secretion of the fibrinogen hexamer, demonstrating the importance of these globular structures in the quality control of fibrinogen biosynthesis.<sup>70</sup> The common A $\alpha$  chain encoded by *FGA* does not contain a globular domain in its C-terminus, but rather a flexible coil, which is likely to tolerate missense mutations better than the globular domain of the  $\beta$  and  $\gamma$  chains. Interestingly, a missense mutation of the A $\alpha$  translation initiation codon (Met1Leu) was recently shown to cause afibrinogenemia.<sup>71</sup>

The large number of mutations identified in patients with congenital afibrinogenemia (►Fig. 3) allows the design of an efficient strategy for mutation detection in new cases.<sup>3</sup> Two common mutations are found in individuals of European origin, both in *FGA*: the IVS4 + 1G > T splice mutation (or c.510 + 1G > T according to the nomenclature guidelines of the Human Genome Variation Society: www.hgvs.org) and the *FGA* 11-kb deletion, both found on multiple haplotypes. In consequence, in all new patients of European origin, the *FGA* IVS 4 + 1 should be the first mutation to be screened. Southern blot or polymerase chain reaction analysis<sup>72</sup> of the *FGA* 11-kb deletion should also be performed, first, because it is the next most common mutation in patients of



**Fig. 3** Fibrinogen gene mutations accounting for afibrinogenemia and hypofibrinogenemia. Mutations are listed here only if the causative nature has been demonstrated by functional analysis or if the type of mutation identified leaves no doubt that it is indeed responsible for the fibrinogen deficiency. References for the original publications can be found at [www.geht.org](http://www.geht.org). Mutations identified in homozygosity or compound heterozygosity in afibrinogenemic patients are in normal font, and those identified in heterozygosity in hypofibrinogenemic individuals are in italic font. Dys- and hypodysfibrinogenemia mutations are not shown. Mutation numbering is based on the cDNA sequences (Genbank entry M64982 for *FGA*, M64983 for *FGB*, and M10014 for *FGG*) with nucleotides numbered from the A in the ATG initiator codon, according to Human Genome Variation Society guidelines. Large deletions are indicated by arrows. The color scale reflects the number of distinct mutations in exons or intron-exon junctions: light blue: 1–6, medium blue: 7–12, and dark blue: > 12 mutations. New mutations identified and appearing in literature since 2009 are shown in red.<sup>3</sup>

European origin, and second, because of the risk of diagnostic error: a nonconsanguineous patient who seems to be homozygous for a mutation in *FGA* exons 2–6 may in reality be a heterozygous carrier of the large 11-kb deletion as we have shown.<sup>72</sup> Given the high incidence of mutations in *FGA*, the other *FGA* exons (starting with exon 5) should then be studied for mutations before screening *FGB* (starting with exon 8) and *FGG*. We apply the same strategy to afibrinogenemic patients of non-European origin for whom recurrent mutations have yet to be identified, but they have a similar spectrum of mutations. Of course, if the patient comes from a geographical region or population for which a mutation has already been

identified, this mutation should be the first to be screened for. In the 3 years following our previous review published in this journal,<sup>3</sup> this approach has allowed us to identify four novel mutations accounting for afibrinogenemia in non-European origin patients, all in *FGA*.<sup>37,71,73,74</sup>

Screening of patients with hypofibrinogenemia can proceed as for afibrinogenemia except for patients presenting with hypofibrinogenemia and ER fibrinogen-positive liver inclusions. Indeed, as indicated in **Table 2**, only four mutations in *FGG* all occurring within or near the “a” hole are known so far to cause hepatic storage disease.<sup>54–57</sup> The majority of dysfibrinogenemias, inherited as a dominant trait, are caused by heterozygous missense mutations in one of the three fibrinogen genes. This topic has been extensively reviewed elsewhere.<sup>4,5</sup> Molecular defects are usually caused by a point mutation that results in the substitution of a single amino acid. These modifications result in alterations in fibrinopeptide release, fibrin polymerization, fibrin cross-linking or fibrinolysis. Only a few patients are homozygous, most of these cases are symptomatic.

Although mutation detection is now relatively easy, it is not always clear whether the identified mutation is the cause of the presenting phenotype. Family studies showing segregation of the mutation with the phenotype, together with allele frequency analysis showing exclusion from the general population and structural correlations are necessary to establish the link between mutation and the disorder. Two mutation “hotspots” are of prime interest in screening for dysfibrinogenemia mutations, that is, residue *FGA* Arg35 (Arg16) in exon 2 which is a part of the thrombin cleavage site in the fibrinogen  $\alpha$  chain and residue *FGG* Arg301 (Arg275) in exon 8, which is important for fibrin polymerization. Mutations at these two sites alone are estimated to account for ~45% of dysfibrinogenemia mutations (from data compiled in [www.geht.org](http://www.geht.org)<sup>75</sup>), but other mutations are common in the surrounding residues. In our laboratory, *FGA* exon 2 and *FGG* exon 8 are the first exons screened in cases of dysfibrinogenemia.

Finally, hypodysfibrinogenemia, which is defined by low levels of a dysfunctional protein, can be caused by different molecular mechanisms. One mechanism is heterozygosity for a single mutation that leads to synthesis of an abnormal fibrinogen chain, which is secreted less efficiently than normal fibrinogen. We recently functionally characterized one such mutation, a 3-bp insertion in *FGA* intron 2 (Fibrinogen Montpellier II) identified in three siblings with hypodysfibrinogenemia. The analysis of precipitated fibrinogen from patient plasma showed that the defect leads to the presence in the circulation of  $\alpha$ -chains lacking knob “A” which is essential for the early stages of fibrin polymerization.<sup>76</sup> Another mechanism is the presence of compound heterozygosity for two different mutations with one mutation responsible for the fibrinogen deficiency (the “hypo phenotype”) and one mutation responsible for the abnormal function of the molecule (the “dys phenotype”), for example, fibrinogen Keokuk<sup>77</sup> and fibrinogen Perth.<sup>78</sup> A different mechanism is found for fibrinogen Leipzig II, since in this case, the common hypofibrinogenemia mutation *FGG* Ala108Gly (Ala82Gly) and

FGG Gly377Ser (Gly351Ser) are located on the same allele.<sup>79</sup> Homozygosity for a single mutation, which allows reduced secretion of a functionally impaired molecule, has been described in fibrinogens Otago<sup>80</sup> and Marburg.<sup>81</sup> Finally, a most interesting case of homozygosity for an *FGB* nonsense mutation Trp353X (Trp323X) accounting for severe hypodysfibrinogenemia was found to be due to heterodisomy or isodisomy of maternal chromosome 4.<sup>82</sup>

### Genotype–Phenotype Correlations

For afibrinogenemia, genotype–phenotype correlations are difficult to establish. First, although in afibrinogenemia, all patients have unmeasurable functional fibrinogen, the severity of bleeding is highly variable amongst patients, even amongst those with the same genotype. Second, there is no clear relationship between the molecular defect and the risk of thrombosis. One possible explanation for the observed variability of clinical manifestations is the existence of modifier genes/alleles: some variants may increase the severity of bleeding, whereas others may ameliorate the phenotype. Such modifiers have yet to be identified; however, common variants predisposing to thrombophilia (e.g., factor V Leiden) most certainly play a role in decreasing the severity of bleeding.

The existence of modifying genes/polymorphisms is also strongly suspected in the previously discussed cases of hypofibrinogenemia associated with fibrinogen inclusion bodies in hepatocytes. Indeed, all individuals heterozygous for one of the four causative mutations identified in *FGG* have hypofibrinogenemia, but not all have fibrinogen aggregates and associated liver disease.

In contrast, several mutations leading to dysfibrinogenemia are predictive of the clinical phenotype,<sup>5</sup> and in such cases, an accurate characterization of the mutant molecule synthesized helps clinicians to understand the pathogenesis of the defect and to predict the clinical outcome. For example the p.Arg573Cys (Arg554Cys) substitution in the A $\alpha$  chain (previously named Chapel Hill III, Paris V and Dusart) predisposes patients to thrombosis.<sup>4,5,83</sup> Here, the impaired fibrinolysis exhibited by this dysfibrinogen seems to be responsible for the thrombotic complications observed. Other examples associated with thrombosis include dysfibrinogens Barcelona III, Haifa I, or Bergamo II due to the common mutation p.Arg301His (Arg275His) in the  $\gamma$  chain and Cedar Rapids I due to Arg301Cys (Arg275Cys). Interestingly, only patients heterozygous for both factor V Leiden and *FGG* p.Arg301His (Arg275His) substitutions were symptomatic, suggesting that this mutation results in thrombosis in combination with another defect.<sup>84</sup> Coexistence of dysfibrinogenemia due to Arg275His with Factor V Leiden was also recently described in a thrombotic patient from Crete.<sup>85</sup> On the contrary, several dysfibrinogenemias, particularly those caused by mutations in the amino-terminal region of the A $\alpha$  chain, for example, Detroit or Mannheim I, p.Arg38Ser (Arg19Ser) and p.Arg38Gly (Arg19Gly), respectively, are associated with bleeding. These examples illustrate how the knowledge of the causative mutation may help to take precautionary measures.

Current diagnostic tests are appropriate for establishing the diagnosis but clearly additional tests are required for a more accurate prediction of the clinical phenotype of a patient and consequently the appropriate treatment. Recently, it has been shown that global assays such as thromboelastography and thrombin generation test may provide a complementary, and in some cases, a better evaluation of an individual's hemostatic state.<sup>86</sup> Studies have shown that in patients with hemophilia A or B presenting similar plasmatic factor VIII or IX levels, measuring thrombin generation, clot formation, and clot lysis in a global assay may yield significantly different results. Similarly, an in vitro study showed the clinical utility of rotational thromboelastography for monitoring the effects of fibrinogen concentrate therapy in patients with fibrinogen deficiency.<sup>87</sup> This suggests that in patients with inherited fibrinogen disorders, global assays could also be useful for the design of individual therapeutic strategies.

Prenatal diagnosis has already been performed in a few cases.<sup>88</sup> For a disease such as afibrinogenemia, where the first sign of the disease is bleeding after loss of the umbilical cord stump, which in some cases is lethal, the prenatal diagnosis of an affected infant allows treatment to be initiated immediately after birth before the first bleeding manifestation.

### Treatment

Replacement therapy is effective in treating bleeding episodes in congenital fibrinogen disorders. Depending on the country of residence, patients receive fresh frozen plasma (FFP), cryoprecipitate, or fibrinogen concentrates.<sup>89</sup> Fibrinogen concentrate preparation includes safety steps for inactivation/removal of viruses, so concentrates are safer than cryoprecipitate or FFP. Furthermore, more precise dosing can be accomplished with fibrinogen concentrates because their potency is known, in contrast to FFP or cryoprecipitates. However, these products are still useful when no concentrates are available.

Four fibrinogen concentrates are available, namely Clottafact from LFB, les Ulis, France; Fibrinogen HT from Benesis, Osaka, Japan; FibroRAAS from Shanghai RAAS, Shanghai, China; and Haemocomplettan from CSL Behring, Marburg, Germany. Preparation of all these concentrates includes safety steps for inactivation/removal of viruses.

The conventional treatment (treatment on demand) is episodic, in which fibrinogen is administered as soon as possible after onset of bleeding. The other approach (prophylaxis) consists of giving either fibrinogen concentrates from an early age to prevent bleeding and, in case of pregnancy, to prevent miscarriage (primary prophylaxis) or after bleeding to prevent recurrences (secondary prophylaxis). Effective long-term secondary prophylaxis with administration of fibrinogen every 7 to 14 days (particularly after central nervous system bleeds) has been described. The frequency and dose of fibrinogen concentrates should be adjusted to maintain a level above 0.5 g/L.<sup>89</sup>

The UK guidelines on therapeutic products for coagulation disorders<sup>90</sup> provide recommendations about the best

treatment options (dosage, management of bleeding, surgery, and pregnancy as well as prophylaxis). According to these guidelines, in case of bleeding fibrinogen levels should be increased to 1.0 g/L and maintained above this threshold until hemostasis is secure and above 0.5 g/L until wound healing is complete. To increase the fibrinogen concentration of 1 g, a dose of ~50 mg/kg is required. The doses and duration of treatment also vary depending on the type of injury or operative procedure. Again, it is essential to take into consideration the patient's personal and familial history of bleeding and thrombosis.

In theory, prophylactic administration of fibrinogen is the best option for patients with severe fibrinogen deficiencies. However, this option has to be counter-balanced with the possible transmission of infectious agents, allergic reactions, venous access problems, development of inhibitors, risk of thrombotic complications, and cost. Furthermore, these patients bleed less than severe hemophiliacs and long asymptomatic periods are not uncommon. In a retrospective survey on patients with afibrinogenemia but also with severe hypofibrinogenemia, the mean annual incidence of bleeding episodes in patients treated on demand was 0.7 (0–16.5); whereas, it was 0.5 (0–2.6) for patients on prophylactic replacement therapy.<sup>29</sup> Compared with severe hemophiliacs (10–15 episodes of bleeding/year without treatment) patients with severe fibrinogen deficiencies seem to bleed less. Another difficulty is to decide on the level of fibrinogen that should be achieved when prophylaxis has been approved. The survey performed by Peyvandi et al<sup>29</sup> showed that preventive doses and intervals vary considerably between physicians: Daily doses of fibrinogen ranged from 0.018 to 0.12 g/kg (mean: 0.06 g/kg). Most patients received weekly doses, and the remaining patients were treated every two weeks or once a month. A fibrinogen level as low as 10% of normal was sufficient to normalize coagulation and platelet adhesion and partially normalize platelet spreading.

The tremendous variability in the clinical course of patients with dysfibrinogenemia makes the clinical management of these patients difficult, and therefore, any treatment should be based on the personal and family history. Indeed, the literature reports relatively complicated cases and this may introduce a bias since patients with clinically more severe diseases will be overrepresented. For example, an Italian study on consecutive patients with congenital fibrinogen disorders showed that the vast majority of patients and their first-degree relatives presenting with dysfibrinogenemia were asymptomatic.<sup>26</sup> With a personal or familial history of thrombosis, thromboprophylaxis and antithrombotic treatments may be proposed after a careful analysis of each particular situation. Long-term management strategies for thrombophilic dysfibrinogenemia are the same as the strategies for patients with recurrent thromboembolism and may include long-term anticoagulation.

In addition to fibrinogen substitution, antifibrinolytic agents may be given, particularly, to treat mucosal bleeding or to prevent bleeding following procedures such as dental extraction. Fibrin glue is useful to treat superficial wounds or following dental extractions. Routine vaccination against

hepatitis as well as a regular surveillance for both the disease and treatment-related complications in a comprehensive care setting is highly recommended.<sup>89</sup>

## Complications of Therapy

In many countries, only FFP or cryoprecipitates are available, which is problematic since the viral inactivation process is in general not as efficient as it is for fibrinogen concentrates. Even if viral inactivation steps are performed, these preparations (particularly FFP) can induce volume overload. There is also a risk of transfusion-related acute lung injury (TRALI) due to cytotoxic antibodies contained in the infused plasma.

Acquired inhibitors after replacement therapy have been reported in only two cases so far.<sup>91,92</sup> It is not clear why afibrinogenemic patients do not develop inhibitors more frequently. One explanation for some cases is that the minute amounts of fibrinogen (that can be detected by the most sensitive immunoassays) are present in the circulation.

Some thrombotic complications temporally associated with fibrinogen replacement therapy have been described.<sup>93</sup> To prevent thrombosis, some clinicians associate small doses of heparin or low-molecular-weight heparin (LMWH) with the administration of fibrinogen. In case of surgery, patients with a thrombotic phenotype should be treated with compression stockings and LMWH. In case of thromboembolic complications, direct anti-Xa or thrombin inhibitors can bind thrombus-bound thrombin, which is not the case with heparin. The successful use of lepirudin has been reported for an afibrinogenemic patient who suffered recurrent arterial thrombosis despite treatment with heparin and aspirin.<sup>94</sup> Thromboembolic complications are always difficult to deal with, as at the same time, it is necessary to give anti-coagulants but also fibrinogen preparations in severe fibrinogen disorders.

Finally, emerging nonviral pathogens such as the prion responsible for variant Creutzfeldt-Jacob disease must be considered, even if no cases have been documented.

## Treatment in Women: Pregnancy, Delivery, and Menorrhagia

Women with congenital afibrinogenemia are able to conceive and embryonic implantation is normal but the pregnancy usually results in spontaneous abortion at 5 to 8 weeks of gestation unless fibrinogen replacement is given.<sup>95</sup> Maintaining the fibrinogen trough level above 0.6 g/L and if possible over 1.0 g/L is recommended. Lower fibrinogen concentrations (< 0.4 g/L) have proven adequate to maintain pregnancy but not to avoid hemorrhagic complications. Continuous infusion of fibrinogen concentrate should be performed during labor to maintain fibrinogen higher than 1.5 g/L (ideally greater than 2.0 g/L).<sup>52,96</sup> However, high-level replacement during pregnancy should be moderated by the fact that thromboembolic events can occur, particularly with the use of cryoprecipitates, which contain appreciable quantities of factor VIII and von Willebrand factor in addition to fibrinogen. Again, it would be helpful to have a test that helps



to predict those women who really need a substitution and at which interval.

Women with hypofibrinogenemia may also have problems with pregnancy. Those with the lower fibrinogen levels may have the higher risk of miscarriage. Problems of pregnancy vary depending on the type of dysfibrinogenemia.

Menorrhagia is an important problem for women with fibrinogen disorders, particularly in afibrinogenemia. Estrogen-progestagen preparations are useful in case of menorrhagia.<sup>90</sup> Oral iron preparations can be given in cases with associated iron-deficiency anemia.

## Perspectives

Even though the number of cases studied is already quite substantial, research in the field, performed in the clinics, and in the research laboratories is still very active. Every year, a large number of novel mutations continue to be characterized in patients suffering from fibrinogen disorders. Indeed, because our previous review published in 2009 in this journal,<sup>3</sup> additional mutations have been identified for all types of congenital fibrinogen disorders. The combining of the results obtained from genetic, biochemical, and clinical analyses will continue to yield valuable information on the development and course of these diseases as well as on the choice of the most appropriate treatments. Patients with congenital fibrinogen deficiencies deserve better predictive tests for clinical complications and more efficient and available fibrinogen concentrates. The international disparity in product availability is a major problem for all coagulation factor concentrates. It can be hoped that new recombinant technologies will increase the availability and markedly reduce the cost of factor concentrates in the future. A definitive cure of their disease is already feasible through liver transplantation, although obviously this approach cannot be envisaged on a large scale. Gene therapy remains a possibility in the long term.

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