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Mutational epidemiology of congenital fibrinogen disorders

Alessandro Casini[1], Marc Blondon[1], Veronique Tintillier[2], Matthew Goodyer[3], Melike E. Sezgin[4], Adalet M. Gunes[4], Michel Hanss[5], Philippe de Moerloose[6], Marguerite Neerman-Arbez[7]

[1]Division of Angiology and Haemostasis, Faculty of Medicine, University Hospitals of Geneva, Geneva, Switzerland

[2] Institut Hématologie Transfusion, Pôle Biologie Pathologie Génétique, CHRU, Lille, France

[3]Department of Haematology, Institut Central des Hôpitaux, Sion, Switzerland

[4]Uludag University, Department of Pediatric Hematology and Oncology, Bursa, Turkey

[5L]aboratoire d'Hématologie, Hospices Civils de Lyon, CBPE, Lyon, France

[6]Faculty of Medicine, Geneva University Hospitals, Geneva, Switzerland

[7]Department of Genetic Medicine and Development, Faculty of Medicine, University of Geneva, Geneva, Switzerland

Corresponding author

Alessandro Casini

Rue Gabrielle-Perret-Gentil 4, 1211 Genève 14, Switzerland

Alessandro.casini@hcuge.ch

Phone number 0041795535482; Fax number 0041223729891

Short running title: Genetics of congenital fibrinogen disorders

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What is known about this topic?

- Congenital fibrinogen disorders (CFD) include quantitative and qualitative diseases.
- CFD result from mutations in one of the three fibrinogen chains leading to defects of synthesis, assembly and secretion of fibrinogen molecules or to structurally abnormal fibrinogen.

What does the paper add?

- Mutational epidemiology of CFD mutations in a large cohort of probands.
- We confirm that common CFD hotspot mutations are reported worldwide, while a few mutations are clustered in certain regions.
- We report 15 new fibrinogen mutations leading to CFD.
- We propose a step-wise genotyping screening strategy for the genetic diagnosis of CFD, analyzing a minimal number of exons.

Abstract

Background: Numerous mutations in *FGA*, *FGB* or *FGG* lead to congenital fibrinogen disorders (CFD), but their epidemiology is not well characterized. The aim of this study was to evaluate the molecular epidemiology of CFD and to develop a genotyping strategy.

Methods: Genetic data from 266 unrelated CFD patients genotyped at our laboratory and from a CFD open access database (n=1142) were evaluated. We developed a step-wise screening strategy for the molecular diagnosis of CFD and prospectively tested this strategy on 32 consecutive CFD probands.

Results: We identified 345 mutated alleles overall, among 187 heterozygous, 63 homozygous and 16 compound heterozygous individuals. Afibrinogenemia was almost always caused by null mutations (98.6%), mainly in *FGA* (85%). Hypofibrinogenemia was mainly caused by missense mutations of *FGB* or *FGG* (54.2%). Dysfibrinogenemia was almost always caused by heterozygous missense mutations (99.3%) in *FGA* and *FGG*. Hotspot mutations were prevalent among quantitative (33.1%) and qualitative fibrinogen disorders (71.1%). The mutational cluster at our laboratory was similar with that reported in the CFD open access database. The proposed step-wise genetic screening strategy proved efficient in both the development and validation samples for CFD: the screening of *FGA* exons 2, 4, 5 and *FGG* exon 8 and search for the 11kb deletion of *FGA* led to the identification of about 80% of mutated alleles, including 15 new mutations.

Conclusion: The described molecular epidemiology of CFD is complex. The proposed step-wise genetic screening strategy may provide an efficient way to identify causative mutations analyzing a minimal number of exons.

Keywords: afibrinogenemia, congenital; hypofibrinogenemia, congenital; dysfibrinogenemia, congenital; hypodysfibrinogenemia, congenital; mutation.

Introduction

Congenital fibrinogen disorders (CFD) include quantitative disorders (i.e., afibrinogenemia and hypofibrinogenemia) and qualitative disorders (i.e., dysfibrinogenemia and hypodysfibrinogenemia), defined according to the functional and antigenic fibrinogen levels (1-3). Mutations in one of the three fibrinogen chain encoding genes (i.e., *FGA*, *FGB*, *FGG*) lead to defects of synthesis, assembly and secretion of fibrinogen molecules or to structurally abnormal fibrinogen (2).

To date, >250 causative mutations have been recorded in open access on-line databases (e.g., http://site.geht.org/base-fibrinogene/) (4, 5). Null mutations, mainly in *FGA*, appear common in quantitative fibrinogen disorders (4, 6) and missense mutations affecting two hotspot residues in exon 2 of *FGA* or exon 8 of *FGG* are believed to be the most frequent causes of dysfibrinogenemia (7). However, although the worldwide prevalence of CFD may be higher than reported to date (8), the precise mutational burden of the *FGA*, *FGB*, and *FGG* genes is unknown. As a precise knowledge of the prevalence of causative mutations could help to design a more efficient and economic strategy for the genetic diagnosis and prenatal screening, our aims were to describe the genetic epidemiology of a large cohort of patients with CFD and to propose a step-wise screening for genetic diagnosis

Materials and Methods

This study was performed with institutional review board approval and with written informed consent from all patients, in accordance with the Declaration of Helsinki. We conducted a retrospective study of all consecutive CFD probands genotyped in our laboratory from 1996 to March 2017, after having excluded 119 relatives.

Demographic data were collected by standardized case report forms, filled in by the physician in charge of the probands. The country of residence was defined by the country where the patient was living at the time of genetic analysis.

Genetic analysis was performed as previously reported (9). Mutations are described according to the Human Genome Variation Society guidelines. Nucleotide numbering is based on the cDNA sequences from GenBank, entry #M64982 for *FGA*, #M64983 for *FGB* and #M10014 for *FGG*. We developed the step-wise algorithm for genetic diagnosis of CFD empirically, focusing on the most common variants in the initial step. Prospective validation of this algorithm was performed using all probands with CFD who were diagnosed in our laboratory from 4.2017 to 2.2018. Mutational results from the Geneva cohort were also numerically compared with genetic results from the Human Fibrinogen Database. This database records fibrinogen variants published or submitted as abstracts in international congresses. In its 44th release (last update 28.12.2017), it encompasses 1102 cases (197 afibrinogenemia, 244 hypofibrinogenemia, 646 dysfibrinogenemia, 55 hypodysfibrinogenemia), including probands and relatives (5).

Analyses were conducted with descriptive statistics and 95% exact binomial confidence intervals.

Results

Epidemiology

A total of 266 probands were genotyped in our laboratory, which led to the genetic diagnosis of 74 afibrinogenemia, 44 hypofibrinogenemia, 141 dysfibrinogenemia and 7 hypodysfibrinogenemia. Dysfibrinogenemia samples were mostly referred from Europe, while the afibrinogenemia cases came mainly from Africa and Asia. Overall, we identified

345 mutated alleles among 187 heterozygous, 63 homozygous and 16 compound heterozygous carriers (Table 1), including a total of 97 unique mutations.

Almost all cases of afibrinogenemia (98.6%) were due to null mutations (i.e., nonsense mutations, splice site mutations, frameshift mutations, large deletion). Only one patient was compound heterozygous [*FGB* c.139C>T(p.Trp47X) and *FGB* c.1330G>C(Gly444Ser)] (10) and one was homozygous [*FGB* c.895T>C(Tyr299His)] (11) for a missense mutation. Most mutations were identified in *FGA* (85%), including the large deletion 11Kb and the splice site mutation c.510+1G>T corresponding to 12.2% and 23.6% of the mutated alleles of afibrinogenemia. In hypofibrinogenemia, heterozygous missense mutations were more frequent (60.4%) and distributed throughout the three genes. Only 2 patients were homozygous for a missense mutation, *FGB* c.853C>T(p.Arg285Cys) and *FGB* c.1415G>T(p.Gly472Val) (11) respectively. Even though hypofibrinogenemic patients are often heterozygous carriers of alleles leading to afibrinogenemia, less null mutations (39.6%) were identified compared to afibrinogenemia. Three patients (6.8% of hypofibrinogenemic patients) were carriers of mutations leading to a fibrinogen storage disease [i.e., c.1201C>T(p.Arg401Trp) and c.1116_1129+1(del372-376) of *FGG*] (12, 13). Almost all dysfibrinogenemias were due to heterozygous missense mutations (99.3%). The missense mutations c.103C>T(p.Arg35Cys) and c.104G>A(p.Arg35His) of *FGA* exon 2 and c.901C>T(p.Arg301Cys) and c.902G>A(p.Arg301His) of *FGG* exon 8 were identified in 7%, 23.2%, 31% and 9.9% of dysfibrinogenemia, respectively. One patient was homozygous for the frameshift mutation c.491insC. All hypodysfibrinogenemic patients were heterozygous and their causative mutations were mainly found in *FGG*.

The exonic distribution of mutations in our series was similar to that reported in the Human Fibrinogen Database (supplementary Table 1). In afibrinogenemia, most mutations

were identified in *FGA* exon 4 (36.5% in our cohort versus 37.3% in the Human Fibrinogen Database) and exon 5 (28.8% versus 39.4%). In dysfibrinogenemia, we identified 87.9% and 88.9% of mutations in exon 2 of *FGA* and exon 8 of *FGG*, compared with 79.4% and 83.9% in the Human Fibrinogen Database. However, it should be noted that the Human Fibrinogen Databases includes also relatives and several of the cases from our series.

In regard to the place of residence, we found no systematic difference in the prevalence of CFD causative mutations. The hotspot mutations (i.e., the 11kb deletion, the c.510+1G>T, the c.103C>T and the c.104G>A mutations of *FGA* and the c.901C>T and c.902G>A of *FGG*) were identified across all continents. Some causative mutations clustered in specific geographic areas, e.g. the nonsense mutations *FGA* c.285T>A(p.Tyr95X) and *FGA* c.635T>G(p.Leu212X) in Lebanon and *FGG* c.8G>A(p.Trp3X) in Slovakia.

Genetic Diagnosis algorithm

As indicated in Figure 1A, the screening for the 11Kb deletion of *FGA* (14) together with the sequencing of *FGA* exons 3, 4, 5 and *FGB* exons 2, 6, 8 and *FGG* exon 8 diagnosed 82.6% of mutated alleles in quantitative fibrinogen disorders. Sequencing of *FGA* exon 2 and *FGG* exon 8 identified 83.9% of causative mutations for dysfibrinogenemia. Screening of *FGA* exons 2, 4, 5 and *FGG* exon 8 and search for the 11kb deletion of *FGA* identified 80% of mutated alleles of CFD. Based on these findings, we developed a step-wise strategy for the genetic analysis of new cases of CFD, shown in Figure 1B. In quantitative fibrinogen disorders, 53.0% (95%CI 43.5-62.4) of patients were diagnosed in step 1, 20.9% of patients (95%CI 13.9-29.4) in step 2, and genotyping of the remaining exons remained necessary in 26.1% of cases (95%CI 18.3-35.1). In qualitative fibrinogen disorders, *FGA* exon 2 and *FGG* exon 8 screening yielded a full genetic diagnosis in 85.8% of patients (95%CI 79.1-91.0). The

step 2 diagnosed an extra 8.8% (95%CI 4.8-14.6), leaving only 5.4% (95%CI 2.4-10.4) with a need for further genotyping.

Prospective assessment of diagnosis algorithm and new variants

We tested this step-wise strategy on 32 consecutive CFD probands referred to our laboratory between April 2017 and February 2018 (Table 2). Similar to the retrospective cohort, the mean age at diagnosis was 25.7 years (2.6 years for afibrinogenemic patients) and samples originated more frequently from Europe (75%).

In qualitative CFD, step 1 yielded a diagnosis in 77% (95% CI 56.6-99.1), with an incremental diagnosis in 11% (95%CI 72.8-100) for both step 2 and step 3. In quantitative CFD, step 1 allowed the identification of 30.8% (95%CI 17.4-59.8), step 2 30.8% (95%CI 30.9-92.1) and step 3 the remaining 38.5% of cases.

We identified 36 mutated alleles leading to 4 afibrinogenemia, 10 hypofibrinogenemia, 15 dysfibrinogenemia and 3 hypodysfibrinogenemia. All afibrinogenemia cases were due to null mutations. Missense mutations leading to hypofibrinogenemic patients were mainly clustered in *FGB* (70%). Hotspot mutations were frequently identified in dysfibrinogenemic alleles (60%). Overall, 15 new fibrinogen mutations were discovered, including a missense mutation in *FBG* exon 6 c.854G>T p.Arg285Leu identified in 3 hypofibrinogenemic patients living in distinct Swiss regions, but without any proven familial relationship. Interestingly, a compound heterozygosity of two new mutations (*FGA* exon 2 c.113G>C p.Arg38Thr and *FGG* exon 8 c.905T>A p.Ala353Ser) resulted in a dysfibrinogenemic phenotype. Both missense mutations were described as deleterious by several protein function prediction tools but we were unable to test them in relatives to confirm their causative role.

Regarding the patients' clinical phenotype, major bleeding was reported in afibrinogenemia while hypofibrinogenemic patients suffered mainly from minor bleeding. As expected, most dysfibrinogenemic patients were asymptomatic. Due to the low number of patients, we were not able to establish any genotype-phenotype correlation. Even among carriers of the same mutation, the clinical phenotype was heterogeneous.

Discussion

The genetic results obtained for our large cohort of patients with CFD add information to previous observations on the genetics of these rare conditions (8, 15). We confirm that afibrinogenemia is due to null mutations resulting in defective synthesis, assembly or secretion of fibrinogen (16). By contrast, missense mutations are more frequently identified in hypofibrinogenemia (17). The disproportion between the ratio of null/missense mutations causing hypofibrinogenemia and afibrinogenemia may be explained by dominant negative effects of missense variants in the heterozygous state. Missense mutations leading to hypofibrinogenemia are clustered in the highly conserved COOH-terminal globular domains of the Bβ and γ chains, showing that substitutions in these structures compromise molecular stability, mostly intra-cellularly since typically mutant chains are not found in the patient's circulation (6). In contrast, the COOH-terminal region of the Aα chain corresponding to the flexible αC domain tolerates missense mutations better. We also confirm that the prevalence of *FGA* exon 2 and *FGG* exon 8 mutations is very high in dysfibrinogenemia (18).

Although CFD can be easily diagnosed based on routine coagulation assays including a functional and antigenic fibrinogen assessment, genotyping is important in order to confirm the diagnosis and to provide some correlation with the clinical phenotype (19). Our screening algorithm efficiently yielded a genetic diagnosis while minimizing the number of

exons analyzed in both the retrospective cohort and the small prospective cohort, especially in qualitative CFD. In quantitative CFD, *FGA* exon 4 and 5 should be screened in parallel to the 11 kb *FGA* deletion. Indeed, a non-consanguineous patient who appears to be homozygous for a mutation in *FGA* exons 2-6 may be a heterozygous carrier of this deletion (14). In cases of familial history of idiopathic liver disease and hypofibrinogenemia, *FGG* exons 8 and 9 should be investigated after histologically proven fibrin accumulation in hepatocytes or abnormal liver function tests such as elevated hepatic enzyme levels (12). In qualitative CFD, the genotype should begin with the sequencing of *FGA* exon 2 and *FGG* exon 8. In cases of a personal and unusual familial history of thrombosis, screening of *FGA* exon 5 and *FGB* exon 2 could be of interest (7). Except for these thrombotic-related fibrinogen variants, the genotype-phenotype correlation in dysfibrinogenemia is not strong enough to allow a more specific exon screening based on the clinical phenotype. If the patient is from a region with a known common mutation, the screening should evidently be directed towards this molecular anomaly.

Our study has some limitations. Firstly, next generation sequencing (NGS) technologies allow sequencing of the coding region of the genome at low cost (20) and are of undeniable value for investigation of bleeding disorders of unknown cause. However, in the setting of CFD where the diagnosis is already clear from other laboratory tests, the additional value offered by the NGS appears limited to particular complex cases (e.g., deep intronic mutations or high clinical suspicion despite normal laboratory tests) (21, 22). Secondly, the number of hypodysfibrinogenemic probands was too low to assess the genetic epidemiology of this sub-type of CFD. Even though most of the reported fibrinogen variants have been widely investigated, the pathogenic role of some new mutations has not been completely validated. However, all new genetics variants were predicted to be deleterious

by SIFT analysis, and, when family members were available for analysis, were absent in those without the phenotype. Thirdly, as most of our samples were from European countries, our step-wise testing approach may not be completely generalizable to other continents. In addition, as in previous studies, we were not able to identify any correlation between the genotype and the patients' phenotype due in part to incomplete clinical data from our retrospective cohort

Finally, our cohort may not be representative of the global population of CFD, as only patients referred to our laboratory have been included, potentially giving rise to inclusion bias.

In conclusion, data from this large cohort of CFD suggest low variability in allelic distribution apart from specific mutation clusters. Our proposed diagnostic strategy is efficient, yielding a full genetic diagnosis in most CFD patients with few genotyped exons.

Addendum

A. Casini, P. de Moerloose, M. Neerman-Arbez designed the research and wrote the paper; Casini and M. Blondon extracted and analyzed the data; A. Casini and M. Neerman-Arbez performed genetic analyses; M. Hanss provided data from the Human Fibrinogen Database; V. Tintillier, M. Goodyer, A. Meral, M. Evim provided genetic samples and patients information (more than two patients in the prospective part of the study). All authors provided critical revisions to the manuscript.

Disclosure of Conflicts of Interest

A. Casini reports research grants from CSL Behring, grants from NovoNordisk, non-financial support from Bayer, outside the submitted work

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Table 1. Demographic and genetic characteristics

	Overall (n=266)	Afib (n= 74)	Hypo (n= 44)	Dysf (n= 141)	HDysf (n= 7)
Demographic					
Men, n (%)	100 (37.6)	35 (47.3)	10 (22.7)	54 (38.3)	1 (14.3)
Age at diagnosis, years, mean (SD)	22.6 (19.22)	3 (4.3)	22.6 (14.9)	32.8 (17.5)	23.4 (10)
Residence					
Europe, n (%)	213 (80.1)	44 (59.5)	33 (75)	130 (92.2)	6 (85.7)
America, n (%)	11 (4.1)	6 (8.1)	3 (6.8)	2 (1.4)	0 (0)
Africa, n (%)	18 (6.7)	9 (12.1)	2 (4.5)	6 (4.3)	1 (14.3)
Asia, n (%)	22 (8.3)	15 (20.3)	5 (11.4)	2 (1.4)	0 (0)
Oceania, n (%)	2 (0.8)	0 (0)	1 (2.3)	1 (0.7)	0 (0)
Status					
Heterozygosity, n (%)	187 (70.3)	0 (0)	40 (90.9)	140 (99.3)	7 (100)
Homozygosity, n (%)	63 (23.7)	58 (78.3)	4 (9.1)	1 (0.7)	0 (0)
Compound het., n (%)	16 (6)	16 (21.7)	0 (0)	0 (0)	0 (0)
FGA, n (%)*	207 (60.0)	126 (85)	13 (27.1)	66 (46.5)	2 (28.6)
Missense mutations, n (%)	64 (18.6)	0 (0)	3 (6.2)	61 (43.0)	0 (0)
Nonsense mutations, n (%)	43 (12.5)	37 (25.0)	4 (8.3)	0 (0)	2 (28.6)
Splice site mutations, n (%)	49 (14.2)	43 (29.0)	5 (10.4)	1 (0.7)	0 (0)
Frameshift mutations, n (%)	27 (7.8)	23 (15.5)	0 (0)	4 (2.8)	0 (0)
Large deletions, n (%)	24 (7.0)	23 (15.5)	1 (2.1)	0 (0)	0 (0)
FGB, n (%)*	38 (11.0)	20 (13.5)	13 (27.1)	4 (2.8)	1 (14.3)
Missense mutations, n (%)	16 (4.6)	2 (1.4)	9 (18.8)	4 (2.8)	1 (14.3)
Nonsense mutations, n (%)	12 (3.5)	10 (6.8)	2 (4.2)	0 (0)	0 (0)
Splice site mutations, n (%)	5 (1.4)	4 (2.7)	1 (2.1)	0 (0)	0 (0)
Frameshift mutations, n (%)	5 (1.4)	4 (2.7)	1 (2.1)	0 (0)	0 (0)
Large deletions, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
FGG, n (%)*	100 (29.0)	2 (1.4)	22 (45.8)	72 (50.7)	4 (57.1)
Missense mutations, n (%)	89 (25.8)	0 (0)	17 (35.4)	70 (49.3)	2 (28.6)
Nonsense mutations, n (%)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (14,3)
Splice site mutations, n (%)	7 (2.0)	2 (1.4)	4 (8.3)	0 (0)	1 (14.3)
Frameshift mutations, n (%)	3 (0.9)	0 (0)	1 (2.1)	2 (1.4)	0 (0)
Large deletions, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*: among the mutated alleles (n=345)

Table 2. Variants identified in 32 consecutive cases used for validation of screening algorithm

Gene, exon	c.DNA	Nascent	Status	Biological phenotype	Gender	Age at diagnosis	Clinical phenotype	Residence
FGA,2	c.103C>T	Arg35Cys	Het	Dysf	Female	23	Spontaneous bleeding, essentially menorrhagia	France
FGA,2	c.103C>T	Arg35Cys	Het	Dysf	Female	28	Thrombosis and bleeding, including post-partum haemorrhage	France
FGA,2	c.104G>A	Arg35His	Het	Dysf	Female	20	Asymptomatic	Australia
FGA,2	c.104G>A	Arg35His	Het	Dysf	Female	20	Asymptomatic	France
FGA,2	c.104G>A	Arg35His	Het	Dysf	Female	14	Asymptomatic	France
FGA,2	c.104G>A	Arg35His	Het	Dysf	Female	48	Asymptomatic	Switzerland
FGA,2	c.113G>C*	Arg38Thr	Het^{+}	Dysf	Male	21	Asymptomatic	Belgium
FGA,2	c.114G>T	Arg38Ser	Het	Dysf	Female	36	Thrombosis	France
FGA,3	c.244A>C*	Thr82Pro	Het	Hypo	Male	4	Thrombosis	France
FGA,5	c.541C>T	Arg181X	Homo	Afib	Female	1	Spontaneous bleeding, including cerebral bleeding	Colombia
FGA,5	c.964A>T*	Lys322X	Homo	Afib	Female	1	Spontaneous bleeding, including muscle hematoma	Turkey
FGA,5	c.1112_1143dup*	-	Het	HDysf	Male	47	Asymptomatic	Switzerland
FGB,3	c.402_410del*	-	Het	Dysf	Female	35	Asymptomatic	Switzerland
FGB,6	c.833-1G>C*	-	Het	Hypo	Female	?	NA	Greece
FGB,6	c.833G>A*	Gly278Glu	Het	Hypo	Female	38	Post-traumatic bleeding	France
FGB,6	c.854G>T*	Arg285Leu	Het	Hypo	Male	65	Thrombosis	Switzerland
FGB,6	c.854G>T*	Arg285Leu	Het	Hypo	Female	16	Spontaneous bleeding, essentially menorrhagia	Switzerland
FGB,6	c.845G>T*	Arg285Leu	Het	Hypo	Female	18	Spontaneous bleeding, essentially cutaneous	Switzerland
FGB,8	c.1244+1G>A	-	Homo	Afib	Male	2	Spontaneous bleeding	Turkey
FGB,8	c.1349G>T*	Gly450Val	Het	Hypo	Female	20	Asymptomatic	France

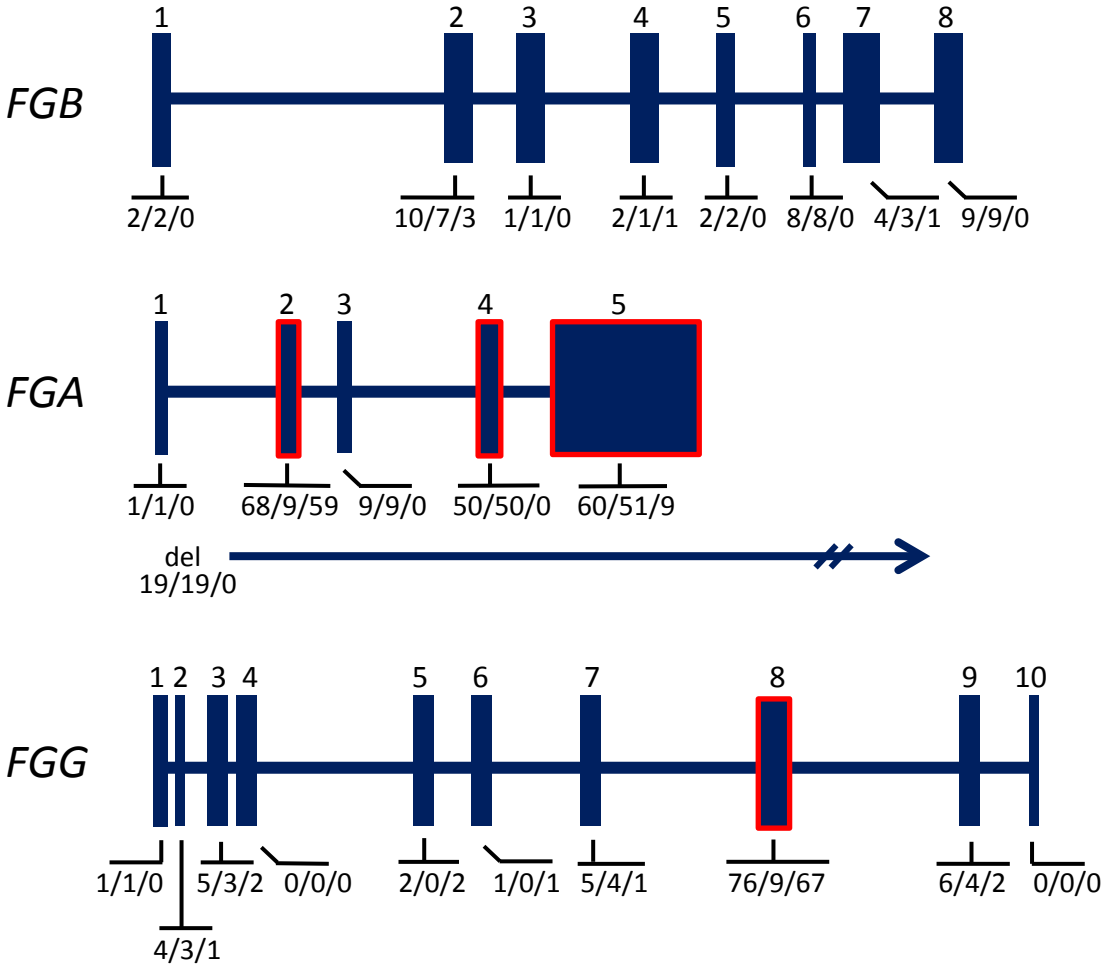
FGB,8	c.1456C>T*	Pro486Ser	Het	Hypo	Female	17	Spontaneous bleeding, essentially menorrhagia	Turkey
FGG,3	c.123+1G>A	-	Homo	Afib	Female	7	Post-traumatic bleeding	USA
FGG,6	c.561G>T*	Trp217Cys	Het	Hypo	Female	53	Asymptomatic	Switzerland
FGG,8	c.902G>A	Arg301His	Het	Dysf	Female	30	Bleeding, including post-partum haemorrhage	France
FGG,8	c.902G>A	Arg301His	Het	Dysf	Male	NA	NA	Argentina
FGG,8	c.902G>A	Arg301His	Het	Dysf	Male	NA	NA	Argentina
FGG,8	c.902G>T	Arg301Leu	Het	Dysf	Female	17	Asymptomatic	France
FGG,8	c.905T>A*	Leu302Gln	Het^{+}	Dysf	Male	21	Asymptomatic	Belgium
FGG,8	c.1057G>T*	Ala353Ser	Het	HDysf	Male	2	Asymptomatic	France
FGG,8	c.1099G>A*	Ala367Thr	Het	HDysf	Female	26	Thrombosis	France
FGG,8	c.1112A>G	Asn371Ser	Het	Hypo	Male	46	Asymptomatic	France
FGG,9	c.1204T>C*	Tyr402Cys	Het	Dysf	Female	70	Asymptomatic	France

*: new fibrinogen variant; +: compound heterozygous. Het: heterozygous; Homo: homozygous; Afib: afibrinogenemia; Dysf: dysfibrinogenemia; Hdysf: hypodysfibrinogenemia; Hypo: hypofibrinogenemia; NA: not available

Figures Legend

Figure 1A. Localization of causative mutations in the fibrinogen-encoding genes. The graphical representation for the major transcript of each gene (*FGB*, *FGA* and *FGG*) is shown with exons, introns and 3'UTRs shown to scale (adapted from the UCSC Genome Browser PDF view of the fibrinogen cluster). Numbers under each exon indicate: total number of mutated alleles/number of mutated alleles leading to afibrinogenemia and hypofibrinogenemia/number of mutated alleles leading to dysfibrinogenemia and hypodysfibrinogenemia located in the corresponding exon and its 3' intron. Exons of particular importance for screening of congenital fibrinogen disorders are indicated boxed in red.

Figure 1B. Suggested strategy for screening of congenital fibrinogen disorders. The term exon includes the exon and its 3'intron. If the patient comes from a geographic region or population for which a mutation has already been identified, screening for this mutation should be included in the first step.


FGB
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1/1/0
2/1/1
2/2/0
8/8/0
4/3/1
9/9/0
FGA
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1/1/0
68/9/59
9/9/0
50/50/0
60/51/9
del
19/19/0
FGG
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1/1/0
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5/4/1
76/9/67
6/4/2
0/0/0