Thrombosis Research 182 (2019) 133–140

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Genetic and clinical characterization of congenital fibrinogen disorders in Polish patients: Identification of three novel fibrinogen gamma chain mutations

Ewa Wypasek[a,b,*], Anna Klukowska[c], Joanna Zdziarska[d], Krystyna Zawilska[e], Jacek Treliński[f], Teresa Iwaniec[g], Andrzej Mital[h], Danuta Pietrys[i], Wojciech Sydor[g], Marguerite Neerman-Arbez[j], Anetta Undas[a,k]

[a] John Paul II Hospital, Krakow, Poland
[b] Faculty of Medicine and Health Sciences, Andrzej Frycz Modrzewski Krakow University, Poland
[c] Department of Pediatrics, Hematology and Oncology, Warsaw Medical University, Warsaw, Poland
[d] Hematology Department, The University Hospital in Krakow, Krakow, Poland
[e] Diagnostic and Treatment Centre INTERLAB, Poznan University of Medical Sciences, Poznan, Poland
[f] Department of Haemostasis Disorders, Medical University of Łodz, Poland
[g] Second Department of Internal Medicine, Jagiellonian University Medical College, Cracow, Poland
[h] Department of Hematology and Transplantology, Medical University of Gdansk, Gdansk, Poland
[i] Department of Oncology and Hematology, Children's University Hospital, Krakow, Poland
[j] Department of Genetic Medicine and Development, Faculty of Medicine, University of Geneva, Geneva, Switzerland
[k] Institute of Cardiology, Jagiellonian University Medical College, Krakow, Poland

ARTICLE INFO

Keywords:
Congenital fibrinogen disorders
Afibrinogenemia
Hypofibrinogenemia
Dysfibrinogenemia
Hypodysfibrinogenemia
Bleeding
Slavic population

ABSTRACT

Introduction: Congenital fibrinogen disorders are poorly explored in Slavic populations. The aim of this study was to characterize the genetic background and clinical manifestations of fibrinogen disorders in the Polish case series.

Materials and methods: In 27 unrelated patients (mean [SD] age, 30.4 [19.2] years, 30% men) with fibrinogen concentration (von Clauss method) < 1.8 g/L, exons and intron-exon junctions of the fibrinogen alpha chain (*FGA*), fibrinogen beta chain (*FGB*), and fibrinogen gamma chain (*FGG*) genes were analyzed using polymerase chain reaction (PCR) amplification followed by sequencing.

Results: At enrollment, 15 (55.6%) and 2 (7.4%) of patients experienced bleeding and thrombotic events, respectively, and the remainder were asymptomatic. The following congenital fibrinogen disorders were identified: 1A. afibrinogenemia, n = 1; 2A. severe hypofibrinogenemia, n = 2; 2B. moderate hypofibrinogenemia, n = 4; 2C. mild hypofibrinogenemia, n = 6; 3A. dysfibrinogenemia, n = 12; 3B. thrombotic related-dysfibrinogenemia, n = 1; 4C. mild hypodysfibrinogenemia, n = 1.

Eight dysfibrinogenemic patients (62%) were carriers of hotspot mutations. Fifteen patients were heterozygous and one (afibrinogenemia) homozygous for known causative mutations. Three new heterozygous mutations were detected, all affecting splicing in *FGG*: fibrinogen Poznan II, a 177 bp deletion eliminating parts of intron 6 and exon 7 in a dysfibrinogenemic woman with recurrent bleeding; fibrinogen Zakopane, (intron 2 acceptor splice site) and fibrinogen Belchatow (intron 1 donor splice site), found in hypofibrinogenemic patients.

During follow-up (median 60, interquartile range 10–60 months), bleeding episodes, mainly menorrhagia and easy bruising were reported in 15 (55.6%) patients. One thromboembolic event was observed.

Conclusion: This study of the largest cohort of Slavic patients with congenital fibrinogen disorders has enabled the identification of 3 new *FGG* mutations and shows a high prevalence of bleeding manifestations with recurrences.

* Corresponding author at: John Paul II Hospital, 80 Prądnicka st., 31-202 Kraków, Poland.
E-mail address: e.wypasek@szpitaljp2.krakow.pl (E. Wypasek).

https://doi.org/10.1016/j.thromres.2019.08.012
Received 18 March 2019; Received in revised form 28 June 2019; Accepted 17 August 2019
Available online 24 August 2019

1. Introduction

Congenital deficiencies of fibrinogen are inherited in an autosomal recessive (afibrinogenemia and hypofibrinogenemia) or dominant (dysfibrinogenemia and hypodysfibrinogenemia) manner with variable penetrance and result from mutations in the fibrinogen alpha chain (*FGA*), fibrinogen beta chain (*FGB*), and fibrinogen gamma chain (*FGG*) genes on chromosome 4q28-q31 [1].

Quantitative disorders include afibrinogenemia and hypofibrinogenemia characterized by absence or decrease of fibrinogen levels, respectively. Qualitative disorders include dysfibrinogenemia (normal quantity of a dysfunctional fibrinogen) and hypodysfibrinogenemia (decreased levels of a dysfunctional fibrinogen) [1–3]. Recently, a new classification of congenital fibrinogen deficiency into four types according to both the clinical phenotype and the fibrinogen levels has been published [2].

The prevalence of afibrinogenemia is estimated at 1 in a million. Hypofibrinogenemia and dysfibrinogenemia are more frequent, however their prevalence is difficult to establish because of the large number of asymptomatic cases [3]. In afibrinogenemia, most patients suffer from major bleeding but can also develop arterial or venous thromboembolism in the presence or absence of fibrinogen replacement [3,4]. The majority of mutations causing afibrinogenemia are null mutations mostly in *FGA* gene [5].

Hypofibrinogenemic patients are frequently heterozygous carriers of afibrinogenemia mutations. In most cases, congenital hypofibrinogenemia results from a mutation of *FGA* or *FGG* genes [1,6]. Hypofibrinogenemic patients with fibrinogen levels above 1 g/L^{-1} are usually asymptomatic [3,7]. In others a more pronounced bleeding phenotype is proportional to the decreased amount of circulating fibrinogen [2].

In dysfibrinogenemia, most individuals are asymptomatic, and are usually discovered incidentally by the prolongation of routine parameters of coagulation [8,9]. Clinical manifestations of congenital dysfibrinogenemia include venous (or rarely arterial) thrombosis (approximately 20% of patients) or bleeding, often occurring during invasive procedures e.g. tooth extraction or spontaneously like epistaxis (25% of patients) [8]. Up to 75% of patients with dysfibrinogenemia of European and Chinese origin are carriers of “hotspot mutations” affecting p.Arg35 in exon 2 of *FGA* gene or p.Arg301 in exon 8 of *FGG* gene. Usually the arginine is replaced by cysteine or histidine which leads to abnormal thrombin cleavage and release of fibrinopeptide A (*FGA* p.Arg35His and p.Arg35Cys) or inaccurate polymerization and end-to-end positioning in the assembly of fibrin monomers (*FGG* p.Arg301His and p.Arg301Cys) [3,9].

Hypodysfibrinogenemia is often symptomatic with mild to moderate bleeding and more likely to lead to thrombosis compared with dysfibrinogenemia [10]. The majority of mutations in hypodysfibrinogenemic patients are due to changes in the C-terminal globular domain of the fibrinogen gamma chain [11]. Patients can either be heterozygous for a single mutation leading to synthesis of an abnormal fibrinogen chain that is secreted less efficiently than normal fibrinogen or compound heterozygous for two different mutations, with one mutation being responsible for the fibrinogen deficiency, and one mutation being responsible for the abnormal function of the molecule [10].

Fibrinogen disorders can cause obstetric complications i.e. mainly spontaneous abortions [3,7].

A few case reports of Polish, Czech and Slovak patients with fibrinogen disorders and known causal mutations have been published so far [12–23]. To the best of our knowledge, we report here on the largest cohort of Polish patients with congenital fibrinogen disorders with their clinical and genetic characterization including long term follow-up data. Three new *FGG* mutations were identified in the course of this study.

2. Patients and methods

A total of 27 unrelated patients with fibrinogen concentration (von Clauss method) < 1.8 g/L on at least 2 separate occasions, were enrolled in the current study between January 2009 and August 2018. The Jagiellonian University Ethical Committee approved the study and all the participants provided their written informed consent. We collected data on clinical manifestations at enrolment and during follow-up.

Major bleeding was defined as any symptomatic bleeding in a critical area or organ (intracranial, intraspinal, intraocular, retroperitoneal, intra-articular, pericardial, intramuscular with the compartment syndrome) or bleeds causing the drop in the hemoglobin levels of at least 20 g/L or leading to two or more red blood cell units transfusion [24]. Clinically relevant non-major bleeding events (CRNMB) were defined as any sign of hemorrhage that did not fulfill major bleeding criteria but met at least one of the following: required medical intervention, led to hospitalization or prompted face to face evaluation, e.g. menorrhagia, prolonged bleeding following tooth extraction [25]. Minor bleeding was defined as every overt bleeding event that does not fulfill the criteria of major or CRNMB bleeding.

The diagnosis of deep vein thrombosis (DVT) was established on the basis of a positive finding of color duplex sonography (the visualization of an intraluminal thrombus in the calf, popliteal, femoral, or iliac vein). The diagnosis of central retinal artery occlusion was based on typical clinical symptoms (abrupt unilateral vision deterioration) and typical appearance of the eye fundus. The diagnosis of cerebral venous sinus thrombosis was established by visualizing sinus stenosis on magnetic resonance angiography. The diagnosis of superficial vein thrombosis (SVT) was made based on the presence of characteristic clinical symptoms and confirmed by compression ultrasound.

Family history of bleeding or thromboembolic events was defined as a self-reported bleeding tendency or presence of thromboembolic events in the first- and second-degree relatives.

The patients were followed up to November 2018. At clinic visits and on telephone contact we collected data on bleeding (based on the ISTH criteria), thromboembolic events as well as obstetric complications and self-reported impaired wound healing.

2.1. Laboratory tests

Blood samples were drawn from an antecubital vein into tubes containing citrate anticoagulant (9,1 of 0.109 M sodium citrate), centrifuged at 2.500*g* at a room temperature for 20 min and processed immediately or stored in aliquots at −80 °C until analysis. Clottable fibrinogen concentrations were estimated by von Clauss method (Multibren U, Siemens; reference range, 1.8–3.5 g/L) and fibrinogen antigen levels were determined nephelometrically (Siemens Healthcare Diagnostics; reference range, 0.19–0.31 g/L). The PT (Thromborel S; reference range, 10.4–13.0 s), aPTT (Pathromtin SL; reference range, 25.9–36.6 s) and Thrombin Time (TT, BC Thrombin Reagent; reference range, < 21 s) were performed on the BCS-XP automated analyzer (Siemens Healthcare, Marburg, Germany).

2.2. Genetic analysis

Whole blood samples for DNA isolation were drawn into K3-EDTA collection tubes and stored in aliquots at –80 °C until processing. DNA was extracted from whole blood or a buffy coat according to the manufacturer's protocol, using Gene MATRIX Quick Blood DNA Purification Kit (Eurex, Gdansk, Poland) and stored at –80 °C until analysis. Exons and intron-exon junctions of the *FGA*, *FGB* and *FGG* genes were analyzed using polymerase chain reaction (PCR) amplification followed by Sanger sequencing. For dysfibrinogenemic patients exon 2 of FGA and exon 8 of *FGG* where analyzed first [9] and when a causative hotspot mutation was identified the remaining exons were not studied.

Mutations were described according to the Human Genome Variation Society guidelines. Nucleotide numbering was based on the complementary DNA sequences from GenBank: entry #M64982 for *FGA* encoding the α-chain, #M64983 for *FGB* encoding the fibrinogen β-chain, and #M10014 for *FGG* encoding the gamma-chain. Amino acid residues and substitutions are numbered from the initiator methionine [9].

2.3. Statistical analysis

The distributions of quantitative variables were analyzed by the Shapiro–Wilk test. Normally distributed variables were compared using one-way analysis of variance (ANOVA) or the *t*-test and were presented as mean (SD). Variables deviating from normal distribution were analyzed by the Kruskal–Wallis ANOVA or Mann–Whitney test and were presented as median (interquartile range) if not otherwise indicated. Qualitative parameters were analyzed by the Pearson $\chi 2$ or 2-tailed Fisher exact test. A P value of < 0.05 was considered significant. Statistical calculations were performed using STATISTICA Version 13.1 (StatSoft, Inc., Tulsa, Oklahoma, United States).

3. Results

The patient characteristics with quantitative and qualitative congenital fibrinogen disorders are shown in Tables 1 and 2, respectively. The mean [SD] age was 30.4 [19.2] years and 8 patients (30%) were male. The dysfibrinogenemic patients comprised 48% (n = 13) of the group. The remaining patients were those with hypofibrinogenemia (44%, n = 12), hypodysfibrinogenemia (4%, n = 1) and afibrinogenemia (4%, n = 1). Functional fibrinogen and antigen levels in patients with dysfibrinogenemia, hypofibrinogenemia and hypodysfibrinogenemia were: 1.19 ± 0.15 g/L and 3.03 ± 0.2 g/L, 1.10 ± 0.15 g/L and 1.19 ± 0.21 g/L, 0.6 ± 0.52 g/L and 1.2 ± 0.63 g/L, respectively. A positive family history of reduced fibrinogen levels was noted in 14 (52%) patients.

At the time of diagnosis, 15 (55.6%) and 2 (7.4%) of patients experienced bleeding and thrombotic events, respectively. In 10 (37%) asymptomatic patients fibrinogen disorders were diagnosed accidentally during routine laboratory tests. Five (18.6%) patients experienced more than two hemorrhagic complications. Two patients (7.4%) experienced bleeding after surgery and two others experienced epistaxis. One patient (3.7%) developed gastrointestinal bleeding and one bleeding after tooth extraction.

Among women with quantitative fibrinogen disorders (n = 8, Table 1), two were pregnant. One (no. 7) received fibrinogen concentrates during the two pregnancies and the deliveries were uneventful and the other (no. 13) was twice pregnant giving birth to healthy children but experiencing postpartum hemorrhage and two miscarriages. Moreover, one woman had a history of 6 miscarriages with persistent vaginal bleedings (no. 1) and one experienced two miscarriages (no. 10).

In women with qualitative fibrinogen deficiency (n = 11, Table 2) six pregnancies were reported. Three of pregnancies were uncomplicated without any treatment due to fibrinogen disorders (no. 1, no. 5 and no. 9). One patient had to receive fibrinogen concentrates during the two pregnancies and the deliveries were uneventful (no. 2). In one case hemorrhagic delivery was observed (no. 4) and one patient experienced spontaneous abortion followed by major hemorrhage (no. 14).

In the entire study group eight (42%) out of 19 women had menorrhagia.

The following congenital fibrinogen disorders using the newest classification of Casini et al. were identified: 1A. afibrinogenemia, n = 1; 2A. severe hypofibrinogenemia, n = 2; 2B. moderate hypofibrinogenemia, n = 4; 2C. mild hypofibrinogenemia, n = 6; 3A. dysfibrinogenemia, n = 12; 3B. thrombotic related-dysfibrinogenemia, n = 1; 4C. mild hypodysfibrinogenemia, n = 1.

Eight dysfibrinogenemic patients (62%) were carriers of hotspot mutations: *FGA* p.Arg35His (n = 3), FGG p.Arg301Cys (n = 2) and FGG p.Arg301His (n = 3). Fifteen patients (10 with hypofibrinogenemia, 4 with dysfibrinogenemia and one with hypodysfibrinogenemia) were found to be heterozygous and one (afibrinogenemia) was homozygous for previously reported causative mutations.

Three new mutations, all in the *FGG* gene were identified, all in heterozygosity. The first, fibrinogen Poznan II, is a 177 bp deletion found in a 33-year woman with dysfibrinogenemia who experienced spontaneous abortion at the age of 33, complicated by genital tract bleeding (Table 2, no. 14). The deletion (del 5716_5892 according to genomic sequence NCBI M10014.1) encompasses the intron 6-exon 7 acceptor splice site and the first 45 codons of *FGG* exon 7. This could either lead to the complete skipping of exon 7, or usage of a new cryptic acceptor splice site since there are several in the vicinity of the deletion. The proband also had a history of menorrhagia, bleeding from the gums and excessive bruising. Her sister, a carrier of the same mutation, did not give birth but had also a history of recurrent bleeding and excessive bruising. The proband and her sister with Poznan II mutation probably inherited it from their father who was not available for genetic analysis. The proband's mother had the fibrinogen levels within the normal range.

The second new mutation, Fibrinogen Zakopane, detected in a hypofibrinogenemic asymptomatic young man, is an acceptor splice-site mutation in *FGG* intron 2: IVS2-2A > C (c.124-2A > C) (Table 1, no. 12). SpliceView analysis predicted that the mutation may create a new acceptor splice site 5 base pairs downstream. If this splice-site is used it would lead to a frameshift in the coding sequence and, if the mutant mRNA is stable enough to be translated, which is unlikely, premature truncation of the gamma chain.

The third new mutation, Fibrinogen Belchatow, was found in a 33-year old woman with hypofibrinogenemia and obstetric history of severe bleeding (Table 1, no. 13). The proband had a strong family history of bleeding in maternal relatives, while the mother herself with fibrinogen levels of 1.75 g/L was asymptomatic. Fibrinogen Belchatow is a donor splice-site mutation in *FGG* intron 1: IVS1 + 5 G > C (c.78 + 5G > C). SpliceView analysis predicts that the mutation completely abolishes the normal donor splice site. This most likely creates an aberrant mRNA retaining intron 1 and encoding 16 aberrant amino acids before a premature stop codon is found in frame.

During follow-up (median 60, interquartile range 10–72 months) the bleeding incidences were detected in 15 (55.6%) of patients, mostly in women (n = 13, 87%). One patient experienced wrist joint bleed without any evident trauma (Table 2, no. 2). She received three times 8 units of cryoprecipitate every 2 days. After that time, the symptoms almost subsided. During follow-up the proband gave birth to two children, as yet asymptomatic (one and two years of age). During the first pregnancy she received 1 g of fibrinogen concentrate once a month for the first trimester, then 1 g every second week in the second trimester and 1 g every third day during the third trimester. The birth was uneventful. During the second pregnancy, she received 1 g of fibrinogen concentrate once a month for the first and second trimesters, and then 1 g every second week in the third trimester. Throughout both pregnancies, the fibrinogen level was 1.1–1.4 g/L.

One patient (Table 2, no. 6) suffered from injury leading to a deep skin wound on the elbow which required surgical sewing. She received 7 units of cryoprecipitate before sewing and 3 g of fibrinogen concentrate before suture removal.

No other major bleeding was recorded. No fatalities were observed. Menorrhagia was reported in 7 women (26%), four with hypofibrinogenemia and three with dysfibrinogenemia.

One woman (Table 1, no. 1) with history of 6 miscarriages received fibrinogen supplementation with therapeutic plasma before minor surgical procedures. One man (Table 1, no. 3) with severe hypofibrinogenemia was treated with cryoprecipitate before tooth extraction.

Table 1
Patients characteristics with quantitative congenital fibrinogen disorders (n = 13).

Patient ID	Sex/age at the time of genetic test	Fibrinogen von Clauss/antigen N: 2.1–4.0 g/L/ 1.8–3.5 g/L	Classification of congenital fibrinogen disorders based on Casini et al. [2]	aPTT/PT N: 25.9–36.6 s/ 10.4–13.0 s	TT N: < 21 s	Type of mutation	Gene/ exon	New/reported	Presentation on admission	Follow-up		Family history of bleeding or thromboembolism
										Duration [months]	Major bleeding/ CRNMB/minor bleeding	
1	F/69	0.93–2.0/1.28–2.0	2B. Moderate hypofibrinogenemia	35.1/16.6	18	c.323C > G, p.Ala108Gly	FGG/4	Reported [13]	Severe bleeding tendency and history of 6 miscarriages with persistent vaginal bleedings	108	0/0/0	1
2	M/25	1.0/1.12	2C. Mild hypofibrinogenemia	33.5/ND	21.1	c.331 A > T, p.Lys111X	FGG/4	Reported (fibrinogen Poznan) [14]	32-hour bleeding episode after tooth extraction, bleeding from minor wounds, epistaxis, easy bruising	108	0/0/easy bruising	1
3	M/29	0.38; 0.39/0.5; 0.6	2A. Severe hypofibrinogenemia	40/17	48.0	c.391T > C, p.Ser131Pro	FGA/4	Reported (fibrinogen Gdansk) [18]	Enormous penile hematoma after penis correction surgery	72	0/0/0	1
4	F/5	0.82/ND	2B. Moderate hypofibrinogenemia	N/N	25	c.331A > T, p.Lys111X	FGG/4	Reported [14]	Laboratory testing prior to adenotomy (without bleed)	60	0/0/easy bruising	1 (farther - nose bleeding)
5	F/19	0.96/ND	2C. Mild hypofibrinogenemia	N/N	22.4	c.323C > G, p.Ala108Gly	FGG/4	Reported [13]	Gastrointestinal bleeding, menorrhagia	60	0/menorrhagia/ 0	1 (sister - menorrhagia, prolonged bleeding after surgery, easy bruising)
6	F/7	1.1/ND	2C. Mild hypofibrinogenemia	N/N	23.5	c. 1330G > A, p.Gly444Ser	FGB/8	Reported [35]	Bleeding after adenotonsillotomy	60	0/0/easy bruising	0
7	F/28	< 0.6/ < 0.15	2A Severe hypofibrinogenemia	30.2/19.1	> 24.0	c.1330G > A, p.Gly444Ser	FGB/8	Reported [35]	Hemorrhagic complications from early childhood, menorrhagia	60	0/menorrhagia/ 0	1
8	F/24	< 0.03/0.04	1A. Afibrinogenemia	180.1/120.1	> 240 s	c. 1330G > A, p.Gly444Ser	FGB/8	Reported [35]	Hemorrhagic complications from 2nd day of life, then menorrhagia	60	0/0/0	0
9	M/17	1.1/1.8	2C. Mild hypofibrinogenemia	36.7/13.4	23.5	c.323C > G, p.Ala108Gly	*FGG*/4	Reported [13]	Accidentally detected prior to surgery	18	0/0/0	1 (VTE in grandparents)
10	F/26	1.29/1.4	2C. Mild hypofibrinogenemia	30.2/13.0	21.5	c.323C > G, p.Ala108Gly	FGG/4	Reported [13]	Menorrhagia, epistaxis, prolonged bleeding after tooth extraction or trauma (eg blood collection)	6	0/menorrhagia/ 0	0
11	M/19	1.6/1.4	2C. Mild hypofibrinogenemia	41.2/14.0	26.9	c.1129G > A, p.Gly377Ser	FGG/8	Reported [10]	Epistaxis from the age of 6	12	0/0/sporadic epistaxis	0
12	M/23	0.93/1.2	2B. Moderate hypofibrinogenemia	34.4/12.9	26.3	IVS2-2 A > C, c.124-2A > C	*FGG*/ intron 2	New (fibrinogen Zakopane)	Detected accidentally	4	0/0/0	1
13	F/33	0.94/1.2	2B. Moderate hypofibrinogenemia	32.7/14.5	31.8	IVS1 + 5 G > C, c.78 + G > C	*FGG*/ intron 1	New (fibrinogen Belchatow)	Postpartum hemorrhage (2×) miscarriages (2×)	8	0/menorrhagia/ 0	1 (bleeding episodes in mother's family)

ND - no data; N - normal range.

Table 2
Patients characteristics with qualitative congenital fibrinogen disorders (n = 14).

Patient ID	Sex/age at the time of genetic test	Fibrinogen von Clauss/antigen N: 2.1–4.0 g/L/ 1.8–3.5 g/L	Classification of congenital fibrinogen disorders based on Casini et al. [2]	aPTT/PT N: 25.9–36.6 s/ 10.4–13.0 s	TT N: < 21 s	Type of mutation	Gene/exon	New/reported	Presentation on admission	Follow-up		Family history of bleeding or thromboembolism
										Duration [months]	Major bleeding/ CRNMB/Minor bleeding	
1	F/21	0.62; 0.56/ 1.16; 1.2	4C. Mild hypodysfibrinogenemia	34/11.4	21.5	c.1052A > T, p.Asn325Ile	FGG/8	Reported (fibrinogen Krakow) [12]	Routine screening during first pregnancy, an appendectomy at the age of 16 complicated by DVT with subsequent post-thrombotic syndrome	108	0/0/0	1
2	F/18	0.86/2.8	3A. Dysfibrinogenemia	30.2/12.9	33.0	c.902 G > A, p.Arg301His	FGG/8	Reported (fibrinogen Zabrze) [15]	Accidentally detected	92	Wrist joint bleed/ 0/0	0
3	M/9	0.7; 0.7/2.56; 2.61	3A. Dysfibrinogenemia	43.3/12	43.1	c.95G > A, p.Gly13Glu	FGA/2	Reported (fibrinogen Krakow II) [16]	Recurrent epistaxis	84	0/0/0	1
4	F/44	2.1/2.29	3A. Dysfibrinogenemia	N/N	22.5	c.124G > A, p.Gly16Ser	*FGG*/2	Reported (fibrinogen Krakow III) [16]	Hemorrhagic delivery and prolonged bleeding following tooth extraction	84	0/0/0	0
5	F/58	1.6; 1.76/3.88	3A. Dysfibrinogenemia	30.5/12.8	31.5	c.104 G > A, p.Arg35His	*FGA*/2	Reported (fibrinogen Krakow IV) [17]	Laboratory testing before scheduled surgery due to significant bleeding history	96	0/0/impaired wound healing, sporadic epistaxis	0
6	F/16	0.57/ND	3A. Dysfibrinogenemia	N/N	31.5	c.902 G > A, p.Arg301His	FGG/8	Reported [15]	Prolonged bleeding following tooth extraction, epistaxis, menorrhagia	60	0/deep skin wound injury/0	Unknown
7	F/20	1.08/ND	3A. Dysfibrinogenemia	N/N	33.8	c.902 G > A, p.Arg301His	FGG/8	Reported [15]	Bleeding after finger injury, menorrhagia	60	0/menorrhagia/0	0
8	F/37	0.6/ND	3A. Dysfibrinogenemia	N/N	46.0	c.901 C > T, p.Arg301Cys	FGG/8	Reported [30]	Accidentally detected prior to surgical removal of varicose veins (without bleeding)	60	0/0/0	0
9	F/42	1.1/3.5	3B. Thrombotic related-dysfibrinogenemia	26.0/13.6	25.4	c.1717C > T, p.Arg573Cys	FGA/5	Reported [36]	Superficial vein thrombosis	60	0/menorrhagia during rivaroxaban treatment/0	1
10	M/67	0.57/3	3A. Dysfibrinogenemia	26.9/13.9	59.6	c.104 G > A, p.Arg35His	*FGA*/2	Reported [9]	Accidentally detected prior to knee surgery and prostatectomy (without complication) at the age 62	10	0/0/0	0
11	F/26	1.31/2.04	3A. Dysfibrinogenemia	30.9/13.1	27.2	c.1330 G > A, p.Gly444Ser	FGB/8	Reported [35]	Cerebral venous and sinus thrombosis during using contraceptives at age of 25	10	0/0/0	0
12	M/23	0.57/3.5	3A. Dysfibrinogenemia	N/N	44.0	c.901 C > T, p.Arg301Cys	FGG/8	Reported [30]	Detected accidentally prior to invasive diagnostics due to unclear cerebrovascular episodes	6	0/0/0	1 (bleeding episodes in father's family)
13	F/81	1.42/4.1	3A. Dysfibrinogenemia	37.1/14.9	33.8	c.104 G > A, p.Arg35His	FGA/2	Reported [9]	Detected accidentally	4	0/0/0	0

(*continued on next page*)

Table 2 (*continued*)

Patient ID	Sex/age at the time of genetic test	Fibrinogen von Clauss/antigen N: 2.1–4.0 g/L/ 1.8–3.5 g/L	Classification of congenital fibrinogen disorders based on Casini et al. [2]	aPTT/PT N: 25.9–36.6 s/ 10.4–13.0 s	TT N: < 21 s	Type of mutation	Gene/exon	New/reported	Presentation on admission	Follow-up		Family history of bleeding or thromboembolism
										Duration [months]	Major bleeding/ CRNMB/Minor bleeding	
14	F/47	1.81/2.6	3A. Dysfibrinogenemia	26.5/11.7	19.1	del 177 bp	FGG/7	New (fibrinogen Poznan II)	Spontaneous abortion at the age of 33 complicated by the genital tract hemorrhage; menorrhagia, bleeding from the gums, easy bruising	12	0/menorrhagia/ easy bruising	1 (mother and sister - menorrhagia, easy bruising)

ND - no data; N - normal range.

Minor bleeding, i.e. epistaxis was reported in one hypofibrinogenemic and one dysfibrinogenemic patients. Excessive bruising characterized 2 patients with hypofibrinogenemia and 2 with dysfibrinogenemia. Impaired wound healing was observed in one patient with dysfibrinogenemia. One thromboembolic event was detected (Table 2, no. 9).

4. Discussion

To the best of our knowledge, this is the largest and most comprehensive study analyzing the genetic background of fibrinogen disorders with long-term follow-up in the Polish population. This Central-Eastern European population mostly consisted of Slavs who arrived at the land of contemporary Poland in the VIth century [26,27]. Since that time no spectacular population movement took place [28]. Clinical phenotypes of our patients were heterogeneous where the same type of mutation was associated with variable clinical presentation.

At the time of diagnosis, 56% of our patients experienced bleeding events, one-third were identified incidentally and those with thrombosis were in the minority. The clinical phenotype of dysfibrinogenemic patients comprising about 50% of our cohort, was similar to German patients [29]. However, compared to other cohort studies on patients from Belgium, Finland, France, Switzerland, United Kingdom, and the United States [9,30], we found a higher prevalence of bleeding than thrombotic events and less patients were asymptomatic on admission. The gender distribution was similar in all studies.

Bleeding events were distributed equally among dys-and hypofibrinogenemic patients. Similarly to a report on English patients [30], among subjects with bleeding, symptoms were typically mild. Hypofibrinogenemic patients with fibrinogen levels above 1 g/L are usually asymptomatic [3,7], however, in our study three out of four hypofibrinogenemic patients with fibrinogen > 1 g/L experienced CRNMB and epistaxis.

Importantly, we identified three novel *FGG* mutations, two manifesting as significant bleeding tendency and positive family history of bleeding. The first one, fibrinogen Poznan II, was present in a woman with dysfibrinogenemia and history of recurrent bleeding at various locations. The second mutation, fibrinogen Zakopane was found in a hypofibrinogenemic asymptomatic man. Other mutations affecting the same acceptor splice site have been reported in homozygosity, in patients with afibrinogenemia [31–33]. The third new mutation, fibrinogen Belchatow was detected in a hypofibrinogenemic women with a positive history of bleeding. Another mutation (IVS1 + 5G > A) affecting this donor splice site has been reported in homozygosity in an afibrinogenemic patient with intracranial bleeding who was born from a consanguineous marriage [34]. In this case minigene analysis in transfected cells indicated retention of intron 1 in the mRNA, a null mutation compatible with the afibrinogenemic phenotype in homozygosity and hypofibrinogenemia in heterozygosity [34].

Other mutations identified here have been previously reported, however to our knowledge, some of them were found in the Slavic population for the first time. For example, we identified the p.Gly444Ser mutation in *FGB* in homozygosity in an afibrinogenemic 24-year-old woman with hemorrhagic complications from early childhood (Table 1, no. 8) diagnosed at day 2 of life based on laboratory findings. She required a standard dose of cryoprecipitate. Due to menorrhagia, she has received fibrinogen concentrate at the beginning of each menstrual period.

The p.Gly444Ser mutation was previously reported in a boy with afibrinogenemia from a British-German family [35]. The proband was a compound heterozygote for 2 mutations in *FGB* gene: an N-terminal nonsense mutation p.Trp47* in exon 2 and the missense mutation p.Gly444Ser in exon 8. In the current study, the p.Gly444Ser variant was also present at heterozygous state in hypo- and dysfibrinogenemic individuals. The clinical manifestations of p.Gly444Ser mutation in these cases were diverse, from bleeding from early childhood to

thrombosis. The woman with severe hypofibrinogenemia (Table 1, no. 7) inherited from her asymptomatic father, experienced a hemorrhagic complication. Another carrier, a 7-year-old girl (Table 1, no. 6) with mild hypofibrinogenemia, developed significant bleeding after adenotonsillotomy. Interestingly, her parents were asymptomatic but both possessed p.Gly444Ser mutation: mother with functional fibrinogen 1,14 g/L at heterozygous state and father with functional fibrinogen 1,9 g/L, at homozygous state. This family is an example where having the same genotype is not related to the same phenotype. We also found the p.Gly444Ser variant at a heterozygous state in a young dysfibrinogenemic woman (Table 2, no. 11) who used hormonal contraception and suffered from cerebral venous sinus thrombosis complicated by stroke. She was treated with enoxaparin at therapeutic doses followed by warfarin and then dabigatran 150 mg bid for 8 months and no recurrent thromboembolism was observed during follow-up.

Another mutation detected for the first time in a Slavic patient was the well characterized fibrinogen Dusart mutation in *FGA* (Paris V, p.Arg573Cys) which is thought to confer an increased risk of thrombosis [36]. This variant has been described in a French family with thromboembolism where two members experienced fatal pulmonary embolism [36] and in a Dutch family with a history of both arterial and venous thrombosis at a young age [37]. In our study, this mutation was found in a young woman (Table 2, no. 9) with unprovoked SVT and her two daughters. The proband was treated with rivaroxaban (20 mg once daily). However, during this treatment severe anemia (Hb < 7 g/dL) due to heavy menstrual bleeding occurred. Similar bleeding episodes also occurred during treatment with dabigatran etexilate (150 mg twice a day). After switching to LMWH the patient did not report similar menstrual bleeding. Very recently, patient experienced distal superficial and deep vein thrombosis after LMWH discontinuation following the ankle joint injury. Now, the proband is on enoxaparin (80 mg daily) and has no adverse events.

One of the proband's daughter had a history of unprovoked proximal DVT of the left leg involving the iliac veins. Initially she was treated with LMWH, then switched to rivaroxaban due to patient preferences (20 mg once daily). Due to bleeding complications that occurred after 12 months of treatment (heavy menstrual bleeds with severe anemia - Hb reduction from 14.8 g/dL to 7.7 g/dL) the patient was switched to vitamin K antagonist (target INR 2.0–3.0) with no further bleeds. No recurrences were noted. The younger proband's daughter (20 years old) had no thromboembolic events.

This finding confirms a strong prothrombotic tendency associated with this mutant fibrinogen supporting the determination of plasma fibrinogen levels in young patients with unprovoked thromboembolism. Such treatment allows the management of anticoagulant therapy and genetic counseling in asymptomatic family members like in other thrombophilias including deficiencies of natural anticoagulants [38,39].

We also identify fibrinogen Praha I (*FGG* p.Gly377Ser) described for the first time in a Polish patient with mild hypofibrinogenemia and recurrent epistaxis (Table 1, no. 11). This mutation was previously reported in a 25-year-old man with abnormal bleeding after several surgical interventions [40]. Given the geographical proximity and historical connections linking two nations, the detection of Fibrinogen Praha I in a Pole is not surprising.

Among our patients with dysfibrinogenemia, 62% were carriers for hotspot mutations which is in agreement with previous reports [3]. The *FGG* p.Arg301His mutation has been identified in asymptomatic patients or in patients with venous or arterial thrombosis [15]. A bleeding tendency has been reported much less frequently. The increased thrombotic risk reported in p.Arg301His carriers may be associated with the formation of relatively dense and poorly lysable fibrin clots [15]. However, in the current study, the p.Arg301His mutation was found in two young women who presented CRNMB at admission and both developed bleeding at 7.5- and 5-year follow-up. It may be speculated that in p.Arg301His carriers with bleeding tendency, the properties of fibrinogen change during several post-translational or post-secretory modifications and/or interactions with other proteins. It also possible that these young patients with bleeding tendency will develop thrombosis later during their lifetime when additional thrombotic risk factors, i.e. oral contraceptives, obesity, injury, coexist [8,9].

Other cases of dysfibrinogenemia resulting from hotspot mutations, *FGG* p.Arg301Cys and *FGA* p.Arg35His, were incidentally detected in asymptomatic patients which is in line with previous reports [30,41].

Interestingly, we detected the second case of fibrinogen Poznan, identified in an asymptomatic 5 year-old girl with moderate hypofibrinogenemia. Her family originated from Poznan indicating on the local nature of this mutation.

During a five-year follow up bleedings occurred in half of our patients and one thromboembolic event was observed. Menorrhagia and easy bruising were the most common incidences which is in line with previous reports [9,29,30]. One major bleed, i.e. a wrist joint bleed was observed in a dysfibrinogenemic patient with the *FGG* p.Arg301His mutation. So far bleeding events are a relatively uncommon presentation for this variant which has been usually found in asymptomatic patients or those with thrombosis [15].

4.1. Study limitation

The patients enrolled in the current study came from clinics from all over Poland. Unfortunately, in some collaborating departments the measurement of the concentration of fibrinogen antigen has been unavailable.

4.2. Conclusions

To our knowledge, we report the largest cohort of Slavic patients with fibrinogen disorders evaluated for the causal genetic background. We found three novel mutations in the *FGG* gene. Like in other populations, hotspot mutations linked to dysfibrinogenemia were observed in most patients. The clinical phenotypes of our patients with fibrinogen disorders, mostly bleeding manifestations with recurrences, were heterogeneous even with the same causative mutation.

Declaration of competing interest

None of the authors declare conflict of interest.

Acknowledgments

We thank Séverine Nolli and Céline Fickentscher for expert technical assistance. This project was funded by a Swiss National Science Foundation grant (#31003A_172864) to M.N.-A. and by the Jagiellonian University Medical College grant (K/ZDS/007717) to A.U.

References

[1] M. Neerman-Arbez, A. Casini, Clinical consequences and molecular bases of low fibrinogen levels, Int. J. Mol. Sci. 19 (2018) e192, https://doi.org/10.3390/ijms19010192.
[2] A. Casini, A. Undas, R. Palla, J. Thachil, P. de Moerloose, Subcommittee on Factor XIII and Fibrinogen, Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH, J. Thromb. Haemost. 16 (2018) 1887–1890, https://doi.org/10.1111/jth.14216.
[3] A. Casini, P. de Moerloose, M. Neerman-Arbez, clinical features and management of congenital fibrinogen deficiencies, Semin. Thromb. Hemost. 42 (2016) 366–374, https://doi.org/10.1055/s-0036-1571339.
[4] M. Nagler, J.A. Kremer Hovinga, L. Alberio, K. Peter-Salonen, H. von Tengg-Kobligk, D. Lottaz, M. Neerman-Arbez, B. Lämmle, Thromboembolism in patients with congenital afibrinogenaemia. Long-term observational data and systematic review, Thromb. Haemost. 27 (2016) 722–732, https://doi.org/10.1160/TH16-02-0082.
[5] M. Neerman-Arbez, P. de Moerloose, C. Bridel, A. Honsberger, A. Schönbörner, C. Rossier, K. Peerlinck, S. Claeyssens, D. Di Michele, R. d'Oiron, M. Dreyfus, M. Laubriat-Bianchin, J. Dieval, S.E. Antonarakis, M.A. Morris, Mutations in the fibrinogen alpha gene account for the majority of cases of congenital

afibrinogenemia, Blood 96 (2000) 149–152.
[6] A. Casini, R. Vilar, Y. Beauverd, D. Aslan, K. Devreese, V. Mondelaers, L. Alberio, C. Gubert, P. de Moerloose, M. Neerman-Arbez, Protein modelling to understand FGB mutations leading to congenital hypofibrinogenaemia, Haemophilia 23 (2017) 583–589, https://doi.org/10.1111/hae.13190.
[7] F. Peyvandi, S. Haertel, S. Knaub, P.M. Mannucci, Incidence of bleeding symptoms in 100 patients with inherited afibrinogenemia or hypofibrinogenemia, J. Thromb. Haemost. 4 (2006) 1634–1637, https://doi.org/10.1111/j.1538-7836.2006.02014.x.
[8] F. Haverkate, M. Samama, Familial dysfibrinogenemia and thrombophilia. Report on a study of the SSC Subcommittee on Fibrinogen, Thromb. Haemost. 73 (1995) 151–161.
[9] A. Casini, M. Blondon, A. Lebreton, J. Koegel, V. Tintillier, E. de Maistre, P. Gautier, C. Biron, M. Neerman-Arbez, P. de Moerloose, Natural history of patients with congenital dysfibrinogenemia, Blood 125 (2015) 553–561, https://doi.org/10.1182/blood-2014-06-582866.
[10] A. Casini, T. Brungs, C. Lavenu-Bombled, R. Vilar, M. Neerman-Arbez, P. de Moerloose, Genetics, diagnosis and clinical features of congenital hypodysfibrinogenemia: a systematic literature review and report of a novel mutation, J. Thromb. Haemost. 15 (2017) 876–888, https://doi.org/10.1111/jth.13655.
[11] D. Vu, M. Neerman-Arbez, Molecular mechanisms accounting for fibrinogen deficiency: from large deletions to intracellular retention of misfolded proteins, J. Thromb. Haemost. 5 (2007) 125–131, https://doi.org/10.1111/j.1538-7836.2007.02465.x.
[12] A. Undas, J. Zdziarska, T. Iwaniec, E. Stepien, A.B. Skotnicki, P. de Moerloose, M. Neerman-Arbez, Fibrinogen Krakow: a novel hypo/dysfibrinogenemia mutation in fibrinogen gamma chain (Asn325Ile) affecting fibrin clot structure and function, Thromb. Haemost. 101 (2009) 975–976.
[13] J. Zdziarska, A. Undas, J. Basa, T. Iwaniec, A.B. Skotnicki, P. de Moerloose, M. Neerman-Arbez, Severe bleeding and miscarriages in a hypofibrinogenemic woman heterozygous for the gamma Ala82Gly mutation, Blood Coagul. Fibrinolysis 20 (2009) 374–376, https://doi.org/10.1097/MBC.0b013e328329f27a.
[14] K. Zawilska, A. Undas, R.J. Fish, L. Molendowicz-Portala, P. de Moerloose, M. Neermen-Arbez, Characterisation of a novel nonsense mutation in FGG (Fibrinogen Poznan) causing hypofibrinogenaemia with a mild bleeding tendency, Thromb. Haemost. 103 (2010) 677–679, https://doi.org/10.1160/TH09-06-0390.
[15] A. Undas, M. Pastuszczak, T. Iwaniec, K. Kapelak, M. Neerman-Arbez, Functional characterisation of plasma fibrin clots in Polish carriers of fibrinogen gammaArg275His mutation (fibrinogen Zabrze), Thromb. Haemost. 104 (2010) 415–417, https://doi.org/10.1160/TH10-02-0114.
[16] D. Pietrys, W. Balwierz, T. Iwaniec, S. Vorjohann, M. Neerman-Arbez, A. Undas, Two different fibrinogen gene mutations associated with bleeding in the same family (A αGly13Glu and γGly16Ser) and their impact on fibrin clot properties: fibrinogen Krakow II and Krakow III, Thromb. Haemost. 106 (2011) 558–560, https://doi.org/10.1160/TH11-02-0102.
[17] J. Zdziarska, T. Iwaniec, A. Undas, A.B. Skotnicki, Bleeding tendency and prolonged wound healing in a patient with A alphaArg16His dysfibrinogenemia: fibrinogen Krakow IV, Thromb. Res. 129 (2012) 532–533, https://doi.org/10.1016/j.thromres.2011.11.015.
[18] A. Mital, A. Undas, M. Neerman-Arbez, A. Hellmann, Fibrinogen Gdansk: hypofibrinogenemia associated with a novel missense mutation in FGA (Ser112Pro), Thromb. Res. 130 (2012) e196–e197, https://doi.org/10.1016/j.thromres.2012.06.021.
[19] R. Kotlín, M. Chytilová, J. Suttnar, T. Riedel, P. Salaj, J. Blatný, J. Santrůcek, P. Klener, J.E. Dyr, Fibrinogen Nový Jicín and Praha II: cases of hereditary Aalpha 16 Arg→Cys and Aalpha 16 Arg→His dysfibrinogenemia, Thromb. Res. 121 (2007) 75–84.
[20] R. Kotlín, B. Blažek, J. Suttnar, M. Malý, J. Kvasnička, J.E. Dyr, Dysfibrinogenemia in childhood: two cases of congenital dysfibrinogens, Blood Coagul. Fibrinolysis 21 (2010) 640–648, https://doi.org/10.1097/MBC.0b013e32833e4284.
[21] R. Kotlín, A. Sobotková, J. Suttnar, P. Salaj, L. Walterová, T. Riedel, Z. Reicheltová, J.E. Dyr, A novel fibrinogen variant—Liberec: dysfibrinogenaemia associated with gamma Tyr262Cys substitution, Eur. J. Haematol. 81 (2008) 123–129, https://doi.org/10.1111/j.1600-0609.2008.01094.x.
[22] T. Simurda, J. Zolkova, Z. Snahnicanova, D. Loderer, I. Skornova, J. Sokol, J. Hudecek, J. Stasko, Z. Lasabova, P. Kubisz, Identification of two novel fibrinogen Bβ chain mutations in two slovak families with quantitative fibrinogen disorders, 29 (2017) 19, https://doi.org/10.3390/ijms19010100 (pii: E100).
[23] D. Vu, P. de Moerloose, A. Batorova, J. Lazur, L. Palumbo, M. Neerman-Arbez, Hypofibrinogenaemia caused by a novel FGG missense mutation (W253C) in the gamma chain globular domain impairing fibrinogen secretion, J. Med. Genet. 42 (2005) e57.
[24] S. Schulman, C. Kearon, Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients, J. Thromb. Haemost. 3 (2005) 692–694, https://doi.org/10.1111/j.1538-7836.2005.01204.x.
[25] S. Kaatz, D. Ahmad, A.C. Spyropoulos, S. Schulman, Subcommittee on Control of Anticoagulation, Definition of clinically relevant non-major bleeding in studies of anticoagulants in atrial fibrillation and venous thromboembolic disease in non-surgical patients: communication from the SSC of the ISTH, J. Thromb. Haemost. 13 (2015) 2119–2226.
[26] P.M. Barford, The Early Slavs: Culture and Society in Early Medieval Eastern Europe, British Museum Press, London, 2001.
[27] K. Rębała, A.I. Mikulich, I.S. Tsybovsky, D. Sováková, Z. Dzupinková, A. Szczerkowska-Dobosz, Z. Szczerkowska, Y-STR variation among Slavs: evidence for the Slavic homeland in the middle Dnieper basin, J. Hum. Genet. 52 (2007) 406–414, https://doi.org/10.1007/s10038-007-0125-6.
[28] A. Wieloucha, Kolonizacja józefińska w galicyjskich Karpatach, PŁAJ 19 (1999) 11–22.
[29] W. Miesbach, I. Scharrer, A. Henschen, M. Neerman-Arbez, S. Spitzer, D. Galanakis, Inherited dysfibrinogenemia: clinical phenotypes associated with five different fibrinogen structure defects, Blood Coagul. Fibrinolysis 21 (2010) 35–40, https://doi.org/10.1097/MBC.0b013e328331e6db.
[30] S.E. Shapiro, E. Phillips, R.A. Manning, C.V. Morse, S.L. Murden, M.A. Laffan, A.D. Mumford, Clinical phenotype, laboratory features and genotype of 35 patients with heritable dysfibrinogenaemia, Br. J. Haematol. 160 (2013) 220–227, https://doi.org/10.1111/bjh.12085.
[31] A. Rottenstreich, A. Lask, L. Schliamser, A. Zivelin, U. Seligsohn, Y. Kalish, Thromboembolic events in patients with severe inherited fibrinogen deficiency, J. Thromb. Thrombolysis 42 (2016) 261–266, https://doi.org/10.1007/s11239-015-1325-0.
[32] M. Neerman-Arbez, P. de Moerloose, A. Honsberger, G. Parlier, B. Arnuti, C. Biron, J.Y. Borg, S. Eber, E. Meili, K. Peter-Salonen, L. Ripoll, C. Vervel, R. d'Oiron, P. Staeger, S.E. Antonarakis, M.A. Morris, Molecular analysis of the fibrinogen gene cluster in 16 patients with congenital afibrinogenemia: novel truncating mutations in the FGA and FGG genes, Hum. Genet. 108 (2001) 237–240.
[33] S. Malaquin, L. Rebibo, C. Chivot, L. Badoux, Y. Mahjoub, H. Dupont, Congenital afibrinogenemia: a case report of a spontaneous hepatic hematoma, Medicine (Baltimore) 95 (2016) e4150, https://doi.org/10.1097/MD.0000000000004150.
[34] R. Asselta, S. Duga, T. Simonic, M. Malcovati, E. Santagostino, P.L. Giangrande, P.M. Mannucci, M.L. Tenchini, Afibrinogenemia: first identification of a splicing mutation in the fibrinogen gamma chain gene leading to a major gamma chain truncation, Blood. 96 (2000) 2496–2500.
[35] D. Vu, P.H. Bolton-Maggs, J.R. Parr, M.A. Morris, P. de Moerloose, M. Neerman-Arbez, Congenital afibrinogenemia: identification and expression of a missense mutation in FGB impairing fibrinogen secretion, Blood 102 (2003) 4413–4415.
[36] J. Soria, C. Soria, P. Caen, A new type of congenital dysfibrinogenaemia with defective fibrin lysis - Dusard syndrome: possible relation to thrombosis, Br. J. Haematol. 53 (1983) 575–586, https://doi.org/10.1182/blood-2003-06-2141.
[37] R. Ramanathan, J. Gram, S. Feddersen, M. Nybo, A. Larsen, J.J. Sidelmann, Dusart Syndrome in a Scandinavian family characterized by arterial and venous thrombosis at young age, Scand. J. Clin. Lab. Invest. 73 (2013) 585–590, https://doi.org/10.3109/00365513.2013.826818.
[38] E. Wypasek, J. Corral, M. Alhenc-Gelas, W. Sydor, T. Iwaniec, M. Celińska-Lowenhoff, D.P. Potaczek, A. Blecharczyk, K. Zawilska, J. Musiał, A. Undas, Genetic characterization of antithrombin, protein C, and protein S deficiencies in Polish patients, Pol. Arch. Intern. Med. 127 (2017) 512–523, https://doi.org/10.20452/pamw.4045.
[39] Z. Bagoly, Uncovering the genetic background of natural anticoagulant deficiencies: time to look behind the scenes, Pol. Arch. Intern. Med. 127 (2017) 465–467, https://doi.org/10.20452/pamw.4069.
[40] R. Kotlín, M. Chytilová, J. Suttnar, P. Salaj, T. Riedel, J. Santrůcek, P. Klener, J.E. Dyr, A novel fibrinogen variant-Praha I: hypofibrinogenemia associated with gamma Gly351Ser substitution, Eur. J. Haematol. 78 (2007) 410–416, https://doi.org/10.1111/j.1600-0609.2007.00838.x.
[41] D.L. Higgins, J.A. Shafer, Fibrinogen Petoskey, a dysfibrinogenemia characterized by replacement of Arg-A alpha 16 by a histidyl residue. Evidence for thrombin-catalyzed hydrolysis at a histidyl residue, J. Biol. Chem. 256 (1981) 12013–12017.