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

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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/\text{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 Bełchatów (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 No 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.
Keywords: congenital fibrinogen disorders, afibrinogenemia, hypofibrinogenemia, dysfibrinogenemia, hypodysfibrinogenemia, bleeding, Slavic population

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 heterozygous, compound heterozygous or homozygous 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 where there is a complete absence of fibrinogen or hypofibrinogenemia characterized by absence or decrease of fibrinogen levels, respectively, proportional decrease of functional and antigenic fibrinogen levels. Qualitative disorders include dysfibrinogenemia (normal quantity of a dysfunctional fibrinogen) decreased functional and normal antigenic fibrinogen levels, and hypodysfibrinogenemia (decreased levels of a dysfunctional fibrinogen) discrepant decrease of functional and antigenic fibrinogen levels) [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] and include large deletions, frameshift mutations and splice site variants [6]. In afibrinogenemic patients of European origin, the most common mutation is a donor splice mutation in intron 4, c.510+1G>T (also known as IVS4+1G>T) [7].

Hypofibrinogenemic patients are frequently heterozygous carriers of afibrinogenemia mutations. In most cases, congenital hypofibrinogenemia results from a mutation of FGA or FGG genes, however missense mutations located in the conserved C-terminal globular D domain of the gamma chain encoded by FGG are also relatively common [1,8].

Hypofibrinogenemic patients with fibrinogen levels above 1 g/L\(^{-1}\) are usually asymptomatic.
In others a more pronounced bleeding phenotype is proportional to the decreased amount of circulating fibrinogen [2]. In some hypofibrinogenemic patients fibrinogen storage disease due to the accumulation of fibrinogen aggregates in the endoplasmic reticulum of hepatocytes are also found [10].

In dysfibrinogenemia, most individuals are asymptomatic, and are usually discovered incidentally by the prolongation of routine parameters of coagulation [11, 128, 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) [118]. 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, 129].

Hypodysfibrinogenemia is often symptomatic with mild to moderate bleeding and more likely to lead to thrombosis compared with dysfibrinogenemia [1310]. The majority of mutations in hypodysfibrinogenemic patients are due to changes in the C-terminal globular domain of the fibrinogen gamma chain, that encompasses several functionally important sites, including the calcium binding (311–336) and polymerisation (374–396) sites [1411]. 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 [1310]. Fibrinogen disorders can cause obstetric complications i.e. mainly spontaneous abortions [3, 97].

A few case reports of Polish, Czech and Slovak patients with fibrinogen disorders and known causal mutations have been published so far [15-26 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.

**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 [2724]. Clinically relevant non-major bleeding events (CRNMB) were defined as any sign of hemorrhage that did not fulfil 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 [2825]. 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.

**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 minutes 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).

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 [129] 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 [129].

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 χ² or 2-tailed Fisher exact test. A P value of less than 0.05 was considered significant. Statistical calculations were performed using STATISTICA Version 13.1 (StatSoft, Inc., Tulsa, Oklahoma, United States).

Results
The patient characteristics with quantitative and qualitative congenital fibrinogen disorders are shown in Table 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 antigenic fibrinogen 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. Two (7.4%) women experienced hemorrhagic events after delivery and another two after miscarriage. 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 haemorrhage (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. She developed elbow joint bleed and 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.

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. No thromboembolic event was detected. One thromboembolic event was detected (Table 2, no. 9).
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. Since that time no spectacular population movement took place. 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. However, compared to other cohort studies on patients from Belgium, Finland, France, Switzerland, United Kingdom, and the United States, 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, among subjects with bleeding, symptoms were typically mild. Hypofibrinogenemic patients with fibrinogen levels above 1 g/L are usually asymptomatic, 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, a 177bp deletion encompassing the intron 6-exon 7 junction was present in a woman with dysfibrinogenemia with and history of recurrent bleeding at various locations. The second mutation, fibrinogen Zakopane was found in a hypofibrinogenemic asymptomatic man. Fibrinogen Zakopane (IVS2-2A>C) affects splicing of intron 2, most likely generating a messenger RNA which will be eliminated by nonsense-mediated decay and therefore not translated. Other mutations affecting the same acceptor splice site have been reported in homozygosity, in patients with afibrinogenemia. The third new mutation, fibrinogen Belchatow was detected in a hypofibrinogenemic woman with a positive history of bleeding, affecting the donor splice site in intron 1 of FGG gene (IVS1+5 G>C). 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
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 [3734]. 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 (patient ID 178, 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. This p.Gly444Ser mutation was previously reported in a boy with afibrinogenemia from a British-German family [3835]. 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 (patient ID 15, Table 1, no. 7) inherited from her asymptomatic father, experienced a hemorrhagic complication. Another carrier, a 7-year-old girl (patient ID 13-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 (patient ID 2011, 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 [3936]. This variant has been described in a French family with thromboembolism where two members experienced fatal pulmonary embolism [3936] and in a young woman (patient ID 12, Table 2, no. 12) with hypofibrinogenemia who was treated successfully with warfarin. Interestingly, the patient’s father was an asymptomatic carrier of the p.Arg573Cys variant.
Dutch family with a history of both arterial and venous thrombosis at a young age [4037]. In our study, this mutation was found in a young woman (patient ID 16, Table 2, no. 9) with unprovoked SVT and her two, so far asymptomatic, 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 [41, 42, 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 (patient ID 22, Table 1, no. 11). This mutation was previously reported in a 25-year-old man with abnormal bleeding after several surgical interventions [43 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 symptomatic patients or in patients with venous or arterial thrombosis [18 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 \[18, 15\]. However, in the current study, the p.Arg301His mutation was found in two young women who presented CRNMB at admission and both developed major 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 \[14, 12, 8, 9\].

Other cases of dysfibrinogenemia resulting from hotspot mutations, \textit{FGG} p.Arg301Cys and \textit{FGA} p.Arg35His, were incidentally detected in asymptomatic patients which is in line with previous reports \[44, 45, 30, 41\]. 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 while no thromboembolic events were observed. Menorrhagia and easy bruising were the most common incidences which is in line with previous reports \[12, 32, 33, 9, 29, 30\]. One major bleed, i.e. an elbow joint bleed and a wrist joint bleed were observed in dysfibrinogenemic patients, both with the \textit{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 \[18, 15\].

\textbf{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.

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 \textit{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.
Acknowledgments

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References


<table>
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<th>Patient ID</th>
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<th>Fibrinogen von Clauss/Antigen N: 1.8-3.5 g/L/0.19-0.31 g/L</th>
<th>Classification of congenital fibrinogen disorders based on Casini et al., 2018</th>
<th>aPTT/PT N:25.9-36.6 s/10.4-13.0 s</th>
<th>TT N:&lt;21 s</th>
<th>Type of mutation</th>
<th>Gene/Exon</th>
<th>New/Reported</th>
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<th>Follow-up</th>
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<td>c.323C&gt;G, p.Ala108Gly</td>
<td>FGG /4</td>
<td>Reported</td>
<td>severe bleeding tendency and history of 6 miscarriages with persistent vaginal bleedings</td>
<td>108</td>
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<td>1.0/1.12</td>
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<td>33.5/22.1</td>
<td>21.1</td>
<td>c.331A&gt;T, p.Lys111X</td>
<td>FGG /4</td>
<td>Reported</td>
<td>32-hour bleeding episode after tooth extraction, bleeding from minor wounds, epistaxis, easy bruising</td>
<td>108</td>
</tr>
<tr>
<td>3</td>
<td>M/29</td>
<td>0.38, 0.39/0.5, 0.6</td>
<td>2A. Severe hypofibrinogenemia</td>
<td>40/17</td>
<td>48.0</td>
<td>c.391T&gt;C, p.Ser131Pro</td>
<td>FGA /4</td>
<td>Reported</td>
<td>enormous penile hematoma after penis correction surgery</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>F/5</td>
<td>0.82/ND</td>
<td>2B. Moderate hypofibrinogenemia</td>
<td>N/N</td>
<td>25</td>
<td>c.331A&gt;T, p.Lys111X</td>
<td>FGG /4</td>
<td>Reported</td>
<td>laboratory testing prior to adenotomy (without bleed)</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>F/19</td>
<td>0.96/ND</td>
<td>2C. Mild hypofibrinogenemia</td>
<td>N/N</td>
<td>22.4</td>
<td>c.323C&gt;G, p.Ala108Gly</td>
<td>FGG /4</td>
<td>Reported</td>
<td>gastrointestinal bleeding, menorrhagia</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>F/7</td>
<td>1.1/ND</td>
<td>2C. Mild hypofibrinogenemia</td>
<td>N/N</td>
<td>23.5</td>
<td>c.1330G&gt;A, p.Gly444Ser</td>
<td>FGB /8</td>
<td>Reported</td>
<td>bleeding after adenotonsillectomy</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>F/28</td>
<td>&lt;0.6/0.15</td>
<td>2A Severe hypofibrinogenemia</td>
<td>30.2/19.1</td>
<td>24.0</td>
<td>c.1330G&gt;A, p.Gly444Ser</td>
<td>FGB /8</td>
<td>Reported</td>
<td>hemorrhagic complications from early childhood, menorrhagia</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>F/24</td>
<td>&lt;0.03/0.04</td>
<td>1A. All fibrinogenemia</td>
<td>180.1/120.1</td>
<td>240 s</td>
<td>c.1330G&gt;A, p.Gly444Ser</td>
<td>FGB /8</td>
<td>Reported</td>
<td>hemorrhagic complications from 2nd day of life, then menorrhagia</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>M/17</td>
<td>1.1/1.8</td>
<td>2C. Mild hypofibrinogenemia</td>
<td>36.7/13.4</td>
<td>23.5</td>
<td>c.323C&gt;G, p.Ala108Gly</td>
<td>FGG /4</td>
<td>Reported</td>
<td>accidentally detected prior to surgery</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>F/26</td>
<td>1.29/1.4</td>
<td>2C. Mild hypofibrinogenemia</td>
<td>30.2/13.0</td>
<td>21.5</td>
<td>c.323C&gt;G, p.Ala108Gly</td>
<td>FGG /4</td>
<td>Reported</td>
<td>menorrhagia, epistaxis, prolonged bleeding after tooth extraction or trauma (eg blood collection)</td>
<td>6</td>
</tr>
<tr>
<td>11</td>
<td>M/19</td>
<td>1.6/1.4</td>
<td>2C. Mild hypofibrinogenemia</td>
<td>41.2/14.0</td>
<td>26.9</td>
<td>c.1129G&gt;A, p.Gly377Ser</td>
<td>FGG /8</td>
<td>Reported</td>
<td>epistaxis from the age of 6</td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>M/23</td>
<td>0.93/1.2</td>
<td>2B. Moderate hypofibrinogenemia</td>
<td>34.4/12.9</td>
<td>26.3</td>
<td>IVS2-2 A&gt;C, c.124-2A&gt;C</td>
<td>FGG / intron 2</td>
<td>New (Fibrinogen Zakopane)</td>
<td>detected accidentally</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>F/33</td>
<td>0.94/1.2</td>
<td>2B. Moderate hypofibrinogenemia</td>
<td>32.7/14.5</td>
<td>31.8</td>
<td>IVS3+5 G&gt;C, c.78+G&gt;C</td>
<td>FGG / intron 1</td>
<td>New (Fibrinogen Belchatow)</td>
<td>postpartum hemorhagia (2x) miscarriages (2x)</td>
<td>8</td>
</tr>
</tbody>
</table>

ND - no data; N - normal range

Table 1. Patients characteristics with quantitative congenital fibrinogen disorders (n=13)
Table 2. Patients characteristics with qualitative congenital fibrinogen disorders (n=14)

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex/Age at the time of genetic test</th>
<th>Fibrinogen von Clauss/Antigen</th>
<th>Classification of congenital fibrinogen disorders based on Casini et al., 2018</th>
<th>aPTT/PT N:25.9-36.6 s/10.4-13.0 s</th>
<th>TT N:&lt;21 s</th>
<th>Type of mutation</th>
<th>Gene/Exon</th>
<th>New/Reported</th>
<th>Presentation on admission</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/21</td>
<td>0.62; 0.56</td>
<td>Mild hypodysfibrinogenemia 34/11.4</td>
<td>21.5</td>
<td>FGG</td>
<td>c.1052A&gt;T, p.Asn325Ile</td>
<td>Reported (Fibrinogen Krakow)</td>
<td>14</td>
<td>routine screening during first pregnancy, an appendectomy at the age of 16 complicated by DVT with subsequent post-thrombotic syndrome</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>F/18</td>
<td>0.86</td>
<td>3A. Dysfibrinogenemia 30.2/12.9</td>
<td>33.0</td>
<td>FGG</td>
<td>c.902 G&gt;A, p.Gly301Glu</td>
<td>Reported (Fibrinogen Zabrze)</td>
<td>15</td>
<td>accidentally detected</td>
<td>92</td>
</tr>
<tr>
<td>3</td>
<td>M/9</td>
<td>0.7; 0.7</td>
<td>Dysfibrinogenemia 43.3/12</td>
<td>43.1</td>
<td>FGA</td>
<td>c.901 C&gt;T, p.Arg301Cys</td>
<td>Reported (Fibrinogen Krakow IV)</td>
<td>30</td>
<td>laboratory testing before scheduled surgery due to significant bleeding history</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>F/44</td>
<td>2.1</td>
<td>3A. Dysfibrinogenemia N/N</td>
<td>22.5</td>
<td>FGG</td>
<td>c.1240G&gt;A, p.Glu413Glu</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>hemorrhagic delivery and prolonged bleeding following tooth extraction</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>F/58</td>
<td>1.6; 1.7</td>
<td>Dysfibrinogenemia 30.5/12.8</td>
<td>31.5</td>
<td>FGA</td>
<td>c.104 G&gt;A, p.Arg35His</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>laboratory testing before scheduled surgery due to significant bleeding history</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
<td>F/16</td>
<td>0.57</td>
<td>3A. Dysfibrinogenemia N/N</td>
<td>31.5</td>
<td>FGG</td>
<td>c.902 G&gt;A, p.Arg301His</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>prolonged bleeding following tooth extraction, epistaxis, menorrhagia</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>F/20</td>
<td>1.06</td>
<td>3A. Dysfibrinogenemia N/N</td>
<td>33.8</td>
<td>FGA</td>
<td>c.902 G&gt;A, p.Arg301Glu</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>bleeding after finger injury, menorrhagia</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>F/37</td>
<td>0.6</td>
<td>3A. Dysfibrinogenemia N/N</td>
<td>46.0</td>
<td>FGA</td>
<td>c.901 C&gt;T, p.Arg301Cys</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>bleeding after finger injury, menorrhagia</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>F/42</td>
<td>1.1/3.5</td>
<td>3B. Thrombotic related-dysfibrinogenemia 26.0/13.6</td>
<td>25.4</td>
<td>FGA</td>
<td>c.1717C&gt;T, p.Arg572Cys</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>superficial vein thrombosis</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>M/67</td>
<td>0.57</td>
<td>3A. Dysfibrinogenemia 26.9/13.9</td>
<td>59.6</td>
<td>FGA</td>
<td>c.104 G&gt;A, p.Arg35His</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>bleeding after knee surgery and prostatectomy (without complication) at the age 62</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>F/26</td>
<td>1.31</td>
<td>3A. Dysfibrinogenemia 30.9/13.1</td>
<td>27.2</td>
<td>FGR</td>
<td>c.1330 G&gt;A, p.Gly444Ser</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>cerebral venous and sinus thrombosis during using contraceptives at age of 25</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>M/23</td>
<td>0.57</td>
<td>3A. Dysfibrinogenemia N/N</td>
<td>44.0</td>
<td>FGA</td>
<td>c.901 C&gt;T, p.Arg301Cys</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>detected accidentally prior to invasive diagnostic tests due to unclear cerebrovascular episodes</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>F/81</td>
<td>1.42</td>
<td>3A. Dysfibrinogenemia 37.1/14.9</td>
<td>33.8</td>
<td>FGA</td>
<td>c.104 G&gt;A, p.Arg35His</td>
<td>Reported (Fibrinogen Krakow II)</td>
<td>16</td>
<td>detected accidentally</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>F/47</td>
<td>1.81</td>
<td>3A. Dysfibrinogenemia 26.5/11.7</td>
<td>19.1</td>
<td>FGG</td>
<td>del 177bp</td>
<td>New (Fibrinogen Poznan II)</td>
<td>12</td>
<td>spontaneous abortion at the age of 33 complicated by the genital tract haemorrhage; menorrhagia, bleeding from the gums, easy bruising</td>
<td>12</td>
</tr>
</tbody>
</table>

ND - no data; N - normal range