RESEARCH LETTER

# A series of Polish patients with congenital fibrinogen disorders: 2 new mutations in fibrinogen gamma chain, Fibrinogen Kostrzyn and Fibrinogen Łódź II

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**Introduction** Congenital fibrinogen disorders are a heterogeneous group of rare abnormalities caused by mutations in 1 of 3 genes encoding fibrinogen polypeptide chains, located on the chromosome 4q28-q31, that is, alpha (*FGA*), beta (*FGB*), or gamma (*FGG*).[1] These disorders can be divided into quantitative fibrinogen deficiencies: afibrinogenemia (complete deficiency of fibrinogen, an autosomal recessive disorder) and hypofibrinogenemia (decreased fibrinogen levels), and qualitative fibrinogen disorders: dysfibrinogenemia (normal levels of dysfunctional fibrinogen, an autosomal dominant disorder) and hypodysfibrinogenemia (decreased levels of dysfunctional fibrinogen).[1,2] The prevalence of afibrinogenemia is estimated at 1:1 000 000, while the worldwide prevalence of hypofibrinogenemia and the qualitative disorders, mostly caused by heterozygous mutations, is much higher.[2] The current classification of congenital fibrinogen deficiencies is based on the clinical phenotype and the level of fibrinogen.[3]

In patients with afibrinogenemia, umbilical stump bleeding is already observed in newborns[4,5] with a further tendency to develop major bleeding.[2] In contrast, symptoms of hypofibrinogenemia, which vary in relation to fibrinogen levels, occur most often after trauma or surgery. However, in women, there is a risk of miscarriages and postpartum hemorrhages.[6] In dysfibrinogenemia, more than 50% of patients are asymptomatic, and the disorder is often detected incidentally, for example, before surgery.[4,7]

Less than 50 cases of Polish patients with congenital fibrinogen disorders and identified causal mutations have been reported until now,[8-10] including the largest cohort of 27 unrelated patients.[11] Here, we present a series of 12 newly diagnosed, consecutive Polish patients with their clinical and genetic characterization, and describe 2 previously unreported mutations in the *FGG* gene.

**Patients and methods** We evaluated 12 unrelated patients with a fibrinogen concentration (von Clauss method) below 1.8 g/l in at least 2 separate measurements, who were referred to the Center for Coagulation Disorders at the John Paul II Hospital, Kraków, Poland. The patients were enrolled between June 2019 and March 2021. We collected data on the clinical manifestations at enrolment and during follow-up.

Major bleeding and clinically relevant nonmajor bleeding (CRNMB) were defined according to the International Society on Thrombosis and Haemostasis criteria.[12] Minor bleeding was defined as any overt bleeding event that did not fulfill the criteria of major bleeding or CRNMB.

An unprovoked episode of venous thromboembolism (VTE) was defined as previously described.[13] The diagnosis of deep vein thrombosis (DVT) was established on the basis of a positive

finding on color duplex sonography.[11] Pulmonary embolism was diagnosed based on the presence of typical symptoms[14] and positive results of computed tomography pulmonary angiography. The diagnosis of cerebral venous sinus thrombosis was established by the presence of typical symptoms and lesions on brain imaging. Family history was regarded as positive if VTE was diagnosed in at least 1 first-degree relative. Risk factors for VTE were also analyzed.

The patients were followed on a 6- to 12-month basis (by means of a visit at the clinic or a telephone interview) until June 2022. New documented thrombotic events, major bleedings or CRNMBs, obstetric complications, and self-reported impaired wound healing were recorded.

All the patients provided written informed consent to participate in the study. Due to the study design, the approval of a Bioethical Committee was not required.

Blood samples were drawn and genetic analysis was performed as previously described.[10] Whole exome sequencing was performed at the Health 2030 Genome Center Sequencing Platform in Geneva, using IDT Research Exome Reagents, multiplexing 12 samples during library preparation. Sequencing was performed on an Illumina HiSeq 4000 (Illumina, San Diego, California, United States), with an estimated mean coverage of 70×. Variant calling was filtered for variants located in a gene panel of 28 genes of the coagulation and fibrinolytic pathways, including *FGA*, *FGB*, and *FGG*.

**Statistical analysis** Continuous variables were presented as mean (SD) or median (interquartile range [IQR]), while qualitative variables were shown as numbers (percentages). Statistical calculations were performed using STATISTICA, Version 13.3 (StatSoft, Inc., Tulsa, Oklahoma, United States).

**Results and discussion** As shown in TABLE 1, the final analysis included 12 probands with quantitative (66.7%) or qualitative (33.3%) congenital fibrinogen disorders, at a mean (SD) age of 33.8 (13.4) years (9 women, 75%), with mean (SD) functional fibrinogen and antigen levels of 1.09 (0.29) g/l and 1.68 (0.18) g/l, respectively.

Among the study group, 6 patients (50.0%) had mild hypofibrinogenemia (2C according to the new classification),[3] 2 (16.7%) had moderate hypofibrinogenemia (2B), and 3 (25.0%) had dysfibrinogenemia (3A). In a single proband (8.3%) moderate hypodysfibrinogenemia (4B) was diagnosed.

At the time of diagnosis, 3 patients (25.0%) experienced bleeding, while 5 individuals (41.7%) had thrombotic events, including 4 cases of DVT and a single case of cerebral venous sinus thrombosis. One patient (TABLE 1; patient no. 7) with no other thrombophilias experienced an unprovoked DVT twice, which is not typical of hypofibrinogenemia but could indicate thrombosis-related dysfibrinogenemia.[2]

Two probands (16.7%) had atypical symptoms, such as painful edema of the arms without any evidence of connective tissue diseases (no antinuclear antibodies; patient no. 1) and allergic skin reactions combined with petechiae on the lower limbs (patient no. 4); the lesions tended to recur with similar intensity. The second patient did not have inhalant allergies, but due to her childhood history of cow's milk protein allergy, she was referred for further diagnosis of contact dermatitis. Furthermore, 2 asymptomatic patients (16.7%) were diagnosed incidentally during routine laboratory investigations. One hypofibrinogenemic and one dysfibrinogenemic female proband (2 out of 9 patients; 22.2%) experienced a miscarriage, both in the third trimester of pregnancy.

Genetic analysis showed that the 2 probands with dysfibrinogenemia were heterozygous carriers of known "hot spot" mutations, that is, *FGA* p.Arg35His and *FGG* p.Arg301Cys. Among the remaining 10 patients, we detected 3 mutations previously described in Slavic populations associated with congenital fibrinogen disorders, namely, *FGG* p.Ala108Gly, *FGB* p.Gly444Ser, and *FGG* p. Lys111X,[8,10] all in heterozygosity. In particular, *FGB* p.Gly444Ser was found in 5 patients with mild or moderate hypofibrinogenemia (TABLE 1; patients no. 5–9) with thromboembolic events or bleeding, which is in line with previous reports indicating that this mutation causes diverse symptoms, from hemorrhage in early childhood to thrombosis.[11]

Two new heterozygous mutations in the *FGG* gene were identified. The first, Fibrinogen Kostrzyn, located in exon 9 (p.Gly392Asp), was identified in a 17-year-old woman (TABLE 1; patient no. 1). This proband with moderate hypodysfibrinogenemia had no bleeding or thrombosis at the time of admission; however, she presented with skin manifestations apparently unrelated to the mutation and the congenital disorders. Her family history (including allergic or autoimmune disease) was negative. Hypodysfibrinogenemia is detected more frequently in women, with various symptoms. Casini et al[15] showed that in these patients bleeding is mainly mild to moderate, with an increased risk during pregnancy and postpartum. Indeed, during follow-up our proband experienced intramuscular and subcutaneous hematomas as well as heavy menstrual bleeding. She received 1 g of fibrinogen concentrate 3 times during menstruation (the blood fibrinogen concentration was approx. 0.5 g/l during the treatment). She gave birth to a baby boy with hypotrophy, who is healthy (now 3 years old). During the pregnancy she was repeatedly hospitalized and received 1 g of fibrinogen concentrate twice a week.

The second new mutation, located in the *FGG* exon 8 (p.Met362Val), Fibrinogen Łódź II, was

**TABLE 1** Characteristics of patients with quantitative or qualitative congenital fibrinogen disorders (continued on the next page)

| Patient ID | Sex/ age, y | Fibrinogen von Clauss/antigen[a] | Classification of congenital fibrinogen disorders[b] | APTT/PT[c] | TT[d] | Type of mutation (all at heterozygous state) | Gene/exon | New/reported | Presentation on admission | Duration of follow-up, mo | Major bleeding/CRNMB/minor bleeding | Thromboembolic events | Family history of bleeding or thromboembolism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F/17 | 0.58/0.9 | 4B. Moderate hypodysfibrinogenemia | 33.4/N | 28.4 | c.1175G>A; p.Gly392Asp | FGG/9 | New (Fibrinogen Kostrzyn) | Accumulation of subcutaneous fluid in the upper limb | 31 | Heavy menstrual bleeding, intramuscular and subcutaneous hematoma/0/0 | 0 | 0 |
| 2 | F/22 | 1.38/1.86 | 3A. Dysfibrinogenemia | N/23.8 | N | c.1084A>G; p.Met362Val | FGG/8 | New (Fibrinogen Łódź II) | Cerebral venous sinus thrombosis, venous stroke (during oral hormonal contraception) | 12 | Heavy menstrual bleeding with a drop in ferritin/0/bleeding gums | 0 | 0 |
| 3 | M/39 | 1.33/1.6 | 2C. Mild hypofibrinogenemia | 27.8/–14.0 | N | c.323C>G; p.Ala108Gly | FGG/4 | Reported | Detected incidentally | 27 | 0/0/0 | 0 | 0 |
| 4 | F/21 | 1.38/1.56 | 2C. Mild hypofibrinogenemia | 33.1/13.4 | 22.8 | c.323C>G; p.Ala108Gly | FGG/4 | Reported | An episode of strong itchiness with petechiae on the lower limbs at the age of 19 | 31 | 0/0/frequent bruising | 0 | 1 (father: indefinite coagulation disorders; father's daughter from the second marriage: epistaxis) |
| 5 | M/31 | 0.89/1.0 | 2B. Moderate hypofibrinogenemia | N/N | 28.6 | c.1330G>A; p.Gly444Ser | FGB/8 | Reported | Prolonged bleeding following tooth extraction, epistaxis | 28 | 0/0/epistaxis (3 times per month) | 0 | 1 |
| 6 | F/46 | 1.19/1.3 | 2C. Mild hypofibrinogenemia | N/N | 25.1 | c.1330G>A; p.Gly444Ser | FGB/8 | Reported | Thrombosis in the vein of the forefoot at the age of 41 | 33 | 0/0/frequent bruising | 0 | 1 (family history of VTE; nonspecific bleeding) |
| 7 | M/61 | 1.18/1.4 | 2C. Mild hypofibrinogenemia | N/N | 24.5 | c.1330G>A; p.Gly444Ser | FGB/8 | Reported | 2 unprovoked DVT episodes at the age of 54 and 55 | 25 | 0/0/0 | 0 | 0 |
| 8 | F/27 | 1.0/1.01 | 2C. Mild hypofibrinogenemia | 26.9/14.7 | 26.9 | c.1330G>A; p.Gly444Ser | FGB/8 | Reported | Miscarriage at 33 weeks (first pregnancy) without placental and fetal pathology | 25 | 0/0/0 | 0 | 1 (mother: DVT, upper venous sinus thrombosis with hemorrhagic stroke in the left hemisphere of the brain after hormonal treatment) |

**TABLE 1** Characteristics of patients with quantitative or qualitative congenital fibrinogen disorders (continued from the previous page)

| Patient ID | Sex/ age, y | Fibrinogen von Clauss/antigen[a] | Classification of congenital fibrinogen disorders[b] | APTT/PT[c] | TT[d] | Type of mutation (all at heterozygous state) | Gene/exon | New/reported | Presentation on admission | Duration of follow-up, mo | Major bleeding/CRNMB/minor bleeding | Thromboembolic events | Family history of bleeding or thromboembolism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | F/21 | 1.0/0.86 | 2C. Mild hypofibrinogenemia | N/14.5 | N | c.1330G>A; p.Gly444Ser | FGB/8 | Reported | Upper extremity VTE | 10 | 0/0/0 | 0 | 1 (father's mother, DVT) |
| 10 | F/34 | 1.19/2.53 | 3A. Dysfibrinogenemia | N/N | 33.5 | c.104G>A; p.Arg35His | FGA/2 | Reported | Asymptomatic | 25 | 0/0/0 | 0 | 1 (father, DVT at the age of 69) |
| 11 | F/32 | 0.54/3.92 | 3A. Dysfibrinogenemia | N/N | 37.0 | c.901C>T; p.Arg301Cys | FGG/8 | Reported | Miscarriage at 29 weeks, spotting from the genital tract | 20 | 0/0/ spotting from the genital tract | 0 | 0 |
| 12 | F/55 | 0.8/1.52 | 2B. Moderate hypofibrinogenemia | N/32.0 | N | c.331A>T; p.Lys111X | FGG/4 | Reported (Fibrinogen Poznań) | FVL; recurrent DVT of both limbs; history of 3 miscarriages | 11 | 0/0/0 | 0 | 1 (mother: FVL, superficial vein thrombosis) |

[a] Normal range, 2.1–4.0 g/l and 1.8–3.5 g/l for fibrinogen and antigen levels, respectively

[b] Based on Casini et al[3]

[c] Normal range, 25.9–36.6 s and 10.4–13.0 s for APTT and PTT, respectively

[d] Normal range <21 s

Abbreviations: APTT, activated partial thromboplastin time; CRNMB, clinically relevant nonmajor bleeding; DVT, deep vein thrombosis; F, female; FVL, Factor V Leiden; M, male; PT, prothrombin time; TT, thrombin time; VTE, venous thromboembolism

found in a 22-year-old woman (TABLE 1; patient no. 2) with dysfibrinogenemia who experienced a massive cerebral venous sinus thrombosis followed by venous stroke during oral hormonal contraception. The patient was treated with enoxaparin (80 mg twice daily) for 4 weeks, followed by acenocoumarol. However, during this treatment, repeated heavy menstrual bleeding was reported, therefore she was switched to non–vitamin K antagonist oral anticoagulants. As shown in previous studies, such a clinical phenotype associated with venous thrombosis occurs in approximately 20% of patients with dysfibrinogenemia.[11]

During a median (IQR) follow-up of 30 (21.5–34.5) months, 8 episodes of bleeding were recorded, including 2 major and 6 minor bleedings, mainly in women. The 2 major bleedings were observed in the patients with Fibrinogen Kostrzyn and Fibrinogen Łódź II mutations. The minor bleedings comprised spotting from the genital tract in a patient with dysfibrinogenemia and a history of a single miscarriage, and in the remaining cases—bruising and epistaxis. No thromboembolic events were detected.

In conclusion, we reported a series of Polish patients with congenital fibrinogen disorders, and identified 2 new mutations in the *FGG* gene detected in women at an early age. The clinical phenotypes in our study group were variable even for patients with the same causative mutation, which is in line with previous reports.[11] Our current case series strongly indicates that molecular diagnosis is an indispensable tool for the identification and management of these rare fibrinogen disorders.

## ARTICLE INFORMATION

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