Dysfibrinogenemia and hypofibrinogenemia – Spectrum of pathogenic variants in Slovak patients

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Introduction. Congenital hypofibrinogenemia (CH) and congenital dysfibrinogenemia (CD) are rare coagulation disorders caused by quantitative or qualitative defects in the fibrinogen gene. The aim of this study was to characterize the genetic background and the clinical manifestations of congenital fibrinogen disorders in the patients from Slovakia registered at the National Haemophilia Centre.
Materials and Methods. Results of genetic analysis of the fibrinogen genes *FGA*, *FGB* and *FGG* using polymerase chain reaction followed by direct sequencing were evaluated in 36 patients.
Results. Molecular-genetic analysis revealed six novel variants – *FGA* c.923_968dup p.(Gly324Lysfs*44) and *FGG* c.1105C>T p.(His369Tyr) were identified in CD patients. In CH patients, in the *FGG* gene c.8G>A p.(Trp3*), c.823G>T p.(Glu275*) and c.323C>A p.(Ala108Asp) variants were detected. In the *FGB* gene c.1427C>T p.(Ser476Leu) was identified.
Conclusion. This study is a positive contribution towards expanding knowledge about genetic variants in patients with congenital fibrinogen disorders.

Key words: dysfibrinogenemia, hypofibrinogenemia, *FGA*, *FGB*, *FGG*

Received: January 21, 2024; Revised: June 18, 2024; Accepted: July 15, 2024; Available online: July 29, 2024
https://doi.org/10.5507/bp.2024.025

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INTRODUCTION

Fibrinogen (FBG) plays a crucial role in haemostasis. Fibrinogen is involved in platelet aggregation and promotes primary haemostasis. The thrombin-mediated conversion of fibrinogen into fibrin and formation of an insoluble fibrin clot represents the final step in the blood coagulation. Fibrinogen also exhibits an anticoagulant effect by binding thrombin, thus limiting its further action in coagulation, and the binding of plasminogen to fibrin plays an important role in promoting fibrinolysis. The fibrinogen molecule is composed of two identical subsets consisting of three homologous polypeptide chains (Aα, Bβ and γ) that are assembled into a hexameric structure. Each subset contains a central E-domain and two distal symmetrical globular D-domains[1,2]. Each fibrinogen polypeptide chain (Aα, Bβ and γ) is encoded by three paralogue genes – *FGA*, *FGB*, *FGG* – clustered on chromosome 4q28-q31 (ref.[3]).

Congenital fibrinogen disorders are rare coagulation disorders which comprise two distinct plasma fibrinogen defects: type I, quantitative deficiencies – afibrinogenemia and hypofibrinogenemia – with absent or reduced concentration of circulating fibrinogen, respectively; and type II, qualitative disorders – dysfibrinogenemia and hypodysfibrinogenemia – caused by structural and functional abnormalities of the fibrinogen molecule. Congenital fibrinogen disorders can be caused by pathogenic variants in any of the three genes, *FGA, FGB* and *FGG,* encoding the fibrinogen chains' synthesis. All variants are reported to international mutation databases. The GFHT database (Groupe Français d'étude sur l'Hémostase et la Thrombose) (ref.[4]) currently comprises a total of 1,215 congenital molecular abnormalities of fibrinogen, out of which 626, 154 and 435 pathogenic variants are localised in *FGA*, *FGB* and *FGG* genes, respectively[4].

Hypofibrinogenemia with an autosomal recessive inheritance is defined by low functional as well as antigenic fibrinogen levels below 1.5 g/L (ref.[5]). In general, genetic analysis in patients with CH identifies pathogenic variants in a heterozygous state, while homozygous or compound heterozygous forms result in afibrinogenemia (null/missense pathogenic variants). Candidate variants associated with CH can affect transcription, mRNA processing, translation and post-translational processing, protein folding and intracellular assembly, stability and secretion of the mature protein. Each of these mechanisms results in a decreased level of fibrinogen in plasma[5]. The bleeding phenotype would be expected to be proportional to reduced fibrinogen levels; however, patients with fibrinogen concentrations above 1.0 g/L (ref.[6]), but often even with lower levels, are usually asymptomatic[6].

Dysfibrinogenemia is characterized by low levels of functional fibrinogen and physiological antigenic fibrinogen concentrations in plasma, while in hypodysfibrinogenemia there is a discrepant decrease of functional and antigenic fibrinogen levels. CD associated with autosomal dominant inheritance is mostly caused by missense pathogenic variants in the coding region of one of the three fibrinogen genes. 85% (ref.[7]) of CD cases are caused by the two most common pathogenic variants within known "hot-spots", in exon 2 of *FGA* and exon 8 of *FGG* genes affecting p.Arg35 and p.Arg301 in the natural fibrinogen protein, respectively. Qualitative modification of the fibrinogen molecule may result in alteration of any of its haemostatic functions including fibrinopeptide release, fibrin polymerization and cross-linking, as well as abnormal fibrinolysis[7]. Clinical features in CD are variable, but most patients are asymptomatic (55%), while some develop bleeding (25%) or thrombosis (20%) (ref.[8,9]).

In Slovakia, all patients with fibrinogen disorders are registered in the National Registry of Congenital Bleeding Disorders which is situated in the National Haemophilia Centre (NHC) at the University Hospital and Medical School of Comenius University in Bratislava. Recently, a comprehensive evaluation of the clinical phenotype in a group of 91 patients (75 with CD and 16 with CH) (ref.[10]) was reported by this centre; however, only a minority of them had been genotyped at that time[10]. The current availability of reliable molecular-genetic tests has increased interest in the study of the genetic background of fibrinogen disorders. Several reports on causal fibrinogen gene variants mostly in individual cases or in small patient cohorts from Central European countries have been published[11-18]. The aim of the current study is to systematically continue the gene analysis and evaluate the genotype-phenotype relationship in patients with fibrinogen disorders registered at the NHC in Bratislava. Here we report the first results of our study.

MATERIALS AND METHODS

Patients and samples

All consecutive patients with congenital fibrinogen disorders visiting NHC between 2019 and 2022 either for diagnosis confirmation, preoperative consultation or a follow up were included into the study. Blood samples in 0.105mol/L tri-sodium citrate and in EDTA were collected by standard method; coagulation tests and immunological analysis of fibrinogen were performed at NHC's Coagulation Lab. EDTA samples were submitted to Institute of Medical Biology, Genetics and Clinical Genetics for genetic analysis by automated direct sequencing of the *FBG* gene. Informed consent was obtained from each patient prior to DNA analysis.

Coagulation tests

Clottable fibrinogen (Fbg:coag) was measured by the Clauss method (Multifibren U, Siemens, Marburg, Germany) and fibrinogen antigen (Fbg:Ag) with an immunonephelometric assay (Siemens, Marburg, Germany). Prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), and functional activity of factors II, V, VII, VIII, IX, and X were measured using standardized methods employing Siemens coagulometers and the reagents, calibrators and factor deficient plasmas from Siemens Healthcare Diagnostic Products GmbH (Marburg, Germany). Fibrinogen/Fibrin degradation products (FDP) were measured with the latex agglutination test (FDP Plasma, Stago, France) and D-dimer with INNOVANCE D-dimer (Siemens, Marburg, Germany).

Molecular-genetic analysis

Molecular-genetic analysis of exons and intron-exon junctions of the fibrinogen genes *FGA*, *FGB* and *FGG* was performed by polymerase chain reaction followed by direct sequencing.

DNA extraction: Genomic DNA was extracted from peripheral blood samples collected into standardized tubes with EDTA according to the manufacturer's protocol, using a commercially available QIAamp DNA Mini Kit (QIAGEN).

Amplification: Primers for *FGA* (Table 1), *FGB* (Table 2) and *FGG* (Table 3) genes were newly designed using Vector NTI Advance® 11.5 and Primer3 v.0.4.0 software. Primer pair specificity was evaluated by Primer-Blast, and the presence of SNPs in designed PCR primer binding sites was evaluated by SNPCheck v3. Larger exons (exon 5 of *FGA*, exon 8 of *FGB*) were separated by overlapping primers to the maximum extent, 700bp. Shorter exons were merged into one PCR product using just one pair of primers (exons 1–2 and 3–4 of *FGG*). All exons of *FGA* (6 exons; 7.5 kb) (ENST00000651975.2), *FGB* (8 exons; 8 kb) (ENST00000302068.9) and *FGG* (10 exons; 8.5 kb) (ENST00000404648.7) and their flanking sequences were amplified using polymerase chain reaction. The reaction mixture contained approximately 100 ng of genomic DNA, 0.3 μmol/L of each primer and PCR master mix (ThermoFisher Scientific) containing dNTPs, buffer and *Taq* DNA polymerase in a final volume of 25 μL. The amplification conditions were: initial denaturation at 95 °C for 5 min followed by 35 cycles of PCR with each cycle consisting of 30 s denaturation at 95 °C, annealing for 30 s at 60 °C (exon 10 of *FGG* at 55 °C), extension for 45 s at 72 °C and the final extension for 10 min at 72 °C. 5μL of the PCR product were used to verify the amplification on 2% agarose gel (100V, 40 min).

Purification: 5 μL of the PCR product was purified using fast alkaline phosphatase (1 μL) and exonuclease I (0.5 μL) before sequencing.

Sequencing: All exons and intron-exon junctions of *FGA*, *FGB* and *FGG* were sequenced on the ABI Prism 3100 Avant Genetic Analyzer using an ABI Prism Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA). Obtained sequences were analysed by Sequencing Analysis Software version 5.4 (Applied Biosystems) and Chromas Lite version 2.6.5, and subsequently compared to Ensembl reference sequences ENST00000651975.2 (*FGA*), ENST00000302068.9 (*FGB*) and ENST00000404648.7 (*FGG*) using Vector NTI Advance 11.5.4 (Invitrogen). All identified pathogenic

Table 1. Primers used for PCR amplification and sequencing of the *FGA* gene (exons 1–6).

Primer labeling	PCR product	Forward primer (5´→ 3´) Reverse primer (5´→ 3´)
FGA 1F	250 bp	ACACAGGACAAAGCCAATGA
FGA 1R		CTGACCTCCAGGCTCTTTGT
FGA 2F	247 bp	CTAACATTGCTGTTGCTCTCTTT
FGA 2R		CAATCAGGTCAGCCTGTGAG
FGA 3F	390 bp	CCAGAGTTTCTCTGCGACCT
FGA 3R		TGTTTCTGTTATAAAGTCAAAGCAGT
FGA 4F	365 bp	TGCTGGCAATTACAGACAAA
FGA 4R		TCTTCAACTGTGGAGTGAGGAA
FGA 5Fa	498 bp	TGGTAGGTCTCCGAGTCAAAA
FGA 5Ra		TTCCAGCACTGCTAGGGTTC
FGA 5Fb	600 bp	TTATGGAACCGGATCAGAGA
FGA 5Rb		TTGTGACCATCAGGACCAAT
FGA 5Fc	688 bp	AAAGGAGATAAAGAGCTCAGGACT
FGA 5Rc		GAGAGTTAAGAAGGAAATGCAAGG
FGA 6Fa	450 bp	TCTGAGCCGTGCCTATCTTT
FGA 6Ra		GAGGAGACTTGGAGGGCATA
FGA 6Fb	482 bp	AAGGGGCTCTGTTCTTAGGG
FGA 6Rb		AAAGAAGACAGAGTGCTCCCATT

Table 2. Primers used for PCR amplification and sequencing of the *FGB* gene (exons 1–8).

Primer labeling	PCR product	Forward primer (5´→ 3´) Reverse primer (5´→ 3´)
FGB 1F	363 bp	CAGCCAACAAGTGAACCAAA
FGB 1R		TCAGAGGTTAACAATTCCATTTCA
FGB 2F	390 bp	CCAGCCAGTTGATGGATCTT
FGB 2R		TGCCATGACTACAGGCTTTCT
FGB 3F	300 bp	CAAATGTCCATGACCCAAATC
FGB 3R		GCAGTCTAACGGTTCCAATTTT
FGB 4F	400 bp	GCTTGGTGATAGCTCAGTGTTT
FGB 4R		TGGTGTGTGAGTTCTTCTGGA
FGB 5F	300 bp	TTCAAAGGTCTATAATAACACACTCCT
FGB 5R		CCTGGCATTTTACGGTATCC
FGB 6F	298 bp	GAATGGACAGGGGATTCAGAT
FGB 6R		CAGGCTTCCAACAATGAATG
FGB 7F	493 bp	TCCTGACAAAAATGTGGAAGC
FGB 7R		TGACAGTAAGTGCCCAGGAA
FGB 8Fa	400 bp	CTTGACCACCGTAGTTCTGTTT
FGB 8Ra		CACACTCACGTCTGCTTGAGA
FGB 8Fb	486 bp	TTTTTGCTCTTCTGTATGTGACAA
FGB 8Rb		CCTTCCTTACAAGTGCAAAGC

variants were confirmed by sequencing with both forward and reverse primers.

Evaluation of novel variants

Novel variants in the *FGA*, *FGB* and *FGG* coding region were analysed and evaluated using web-based tools – MutationTaster (http://www.mutationtaster.org/), PredictSNP (https://loschmidt.chemi.muni.cz/predictsnp/), and Varsome (https://varsome.com/) – to assess their potential pathogenicity according to ACMG criteria.

RESULTS

Clinical and laboratory phenotypes were evaluated in 36 patients with congenital fibrinogen disorders comprising 31 index cases and 5 affected first-degree relatives. In

Table 3. Primers used for PCR amplification and sequencing of the *FGG* gene (exons 1–10).

Primer labeling	PCR product	Forward primer (5´→ 3´) Reverse primer (5´→ 3´)
FGG 1-2F	574 bp	TGCTTCACTAGCCTTTGCAG
FGG 1-2R		CTATTCCTCTGCCCCTCTCC
FGG 3-4F	646 bp	CCCTCATAAATATCATCTGGCACT
FGG 3-4R		CAGGCATAATGTCACTGGGATA
FGG 5F	250 bp	CTCTTCTGAAAGGAATACTTATTTTTG
FGG 5R		CCTTTACATTACTTACTTTGATGAACC
FGG 6F	249 bp	AAGGCTGGCACAGTCTTACC
FGG 6R		ATGGCAGAAACAATGTAGGAA
FGG 7F	300 bp	GCATTCTCTTTACATGCATTGAT
FGG 7R		CATTCCAGGCAATCTTTACAA
FGG 8F	487 bp	AGATCCCTGAGGAGGGTCAG
FGG 8R		TCCACTTCCAGTTTCAAAGAACT
FGG 9F	395 bp	ACTGGCAATGCACTTCGTAA
FGG 9R		AAAAAGGAAGAAACTTTCAGAGAA
FGG 10F	398 bp	TTCCTTGTTTATTACCCCTAAATCA
FGG 10R		TGATGGTTGCCCAATTGTAA

Table 4. Patients with congenital dysfibrinogenemia. Clinical manifestation, laboratory characteristics and identified variants in probands.

Patient No	Family No	Age years	Gender	Gene	Exon	c.DNA	Native protein	Fbg:coag g/L	Fbg:Ag g/L	Clinical manifestation
1	(F1)	54	M	FGA	2	c.104G>A	p.Arg35His	0.70	2.40	asymptomatic
2	(F1)	26	M	FGA	2	c.104G>A	p.Arg35His	0.45	2.90	bleeding
3	(F2)	72	F	FGA	2	c.104G>A	p.Arg35His	0.80	3.60	bleeding
4	(F2)	34	F	FGA	2	c.104G>A	p.Arg35His	1.30	5.20	bleeding
5		8	M	FGA	2	c.104G>A	p.Arg35His	0.40	1.9	asymptomatic
6		29	F	FGA	2	c.104G>A	p.Arg35His	0.96	2.70	asymptomatic
7		8	M	FGA	2	c.104G>A	p.Arg35His	0.65	2.40	asymptomatic
8		34	F	FGA	2	c.104G>A	p.Arg35His	0.45	5.70	bleeding
9		35	F	FGA	2	c.104G>A	p.Arg35His	0.89	2.50	asymptomatic
10		63	M	FGA	2	c.104G>A	p.Arg35His	0.70	3.58	bleeding
11		12	F	FGA	2	c.104G>A	p.Arg35His	0.80	1.70	asymptomatic
12		59	F	FGA	2	c.104G>A	p.Arg35His	1.66	4.90	asymptomatic
13		40	F	FGA	5	**c.923_968dup**	**p.(Gly324Lysfs*44)**	1.53	2.01	asymptomatic
14		33	F	FGG	8	c.901C>T	p.Arg301Cys	0.55	2.70	bleeding
15	(F3)	63	M	FGG	8	c.901C>T	p.Arg301Cys	0.30	2.79	asymptomatic
16**	(F3)	30	M	FGG	8	c.901C>T	p.Arg301Cys	0.3	2.28	bleeding + thrombosis
				FGB	8	/c.1433G>A	p.Arg478Lys[&]			
17**	(F4)	72	M	FGG	8	**c.1105C>T**	**p.(His369Tyr)**	0.40	1.60	thrombosis
				FGB	8	/c.1433G>A	p.Arg478Lys[&]			
18	(F4)	33	F	FGG	8	**c.1105C>T**	**p.(His369Tyr)**	0.58	1.80	asymptomatic

Novel variants are in **bold**.

F, Family with at least one relative; *FGA* transcript ID ENST00000403106.8 (NM_021871.4), *FGB* transcript ID ENST00000302068.9 (NM_005141.5), *FGG* transcript ID ENST00000336098.8 (NM_021870.3); **Digenic variant; [&]Nonsynonymous polymorphism.

a group of 18 patients with congenital dysfibrinogenemia (CD) and a median age of 34 years (range 8–72 years) the levels of functional fibrinogen (Fgb:coag) and fibrinogen antigen (Fbg:Ag) median (range, interquartile range - IQR) were 0.68 g/L (0.3–1.53 g/L; IQR: 0.45–0.96 g/L) and 3.7 g/L (1.6–5.7 g/L; IQR: 2.4–3.6 g/L), respectively. Clinical evaluation showed 10 (55.5%) being asymptomatic individuals, mild bleeding occurred in 7 (38.8%) and thrombotic events in 2 (11.1%) patients, but one patient had symptoms of both.

Table 5. Patients with congenital hypofibrinogenemia. Clinical manifestation, laboratory characteristics and identified variants in probands.

Patient	No of family	Age years	Gender	Gene	Exon	c.DNA	Native protein	Fbg:coag g/L	Fbg:Ag g/L	Clinical manifestation
1		17	F	FGB	4	c.534G>C	p.(Lys178Asn)	1.10	1.15	asymptomatic
2**	(F1)	9	M	FGB	int 6	c.959-13_959-10del	-			
				FGG	4	**/c.323C>A**	**p.(Ala108Asp)**	1.40	1.40	thrombosis
				FGB	8	/c.1433G>A	p.Arg478Lys[&]			
3**	(F1)	13	M	FGB	int 6	c.959-13_959-10del	-			
				FGG	4	**/c.323C>A**	**p.(Ala108Asp)**	1.03	1.1	asymptomatic
				FGB	8	/c.1433G>A	p.Arg478Lys[&]			
4		42	F	FGB	7	c.1102T>C	p.(Tyr368His)	1.27	1.3	bleeding
5		6	M	FGB	7	c.1102T>C	p.(Tyr368His)	0.66	0.9	asymptomatic
6		71	F	FGB	7	c.1102T>C	p.(Tyr368His)	1.09	1.00	bleeding
7		33	F	FGB	7	c.1102T>C	p.(Tyr368His)	0.97	1.40	thrombosis
8		33	F	FGB	8	**c.1427C>T**	**p.(Ser476Leu)**	0.93	1.07	asymptomatic
9		28	F	FGG	1	**c.8G>A**	**p.(Trp3*)**	0.80	0.90	bleeding
10		19	M	FGG	1	**c.8G>A**	**p.(Trp3*)**	0.85	1.1	asymptomatic
11		39	M	FGG	4	c.323C>G	p.Ala108Gly	1.45	1.50	asymptomatic
12		41	F	FGG	4	c.323C>G	p.Ala108Gly	1.00	1.10	bleeding
13		22	M	FGG	4	c.323C>G	p.Ala108Gly	1.05	1.20	asymptomatic
14		14	M	FGG	4	c.323C>G	p.Ala108Gly	1.34	0.82	asymptomatic
16		23	F	FGG	7	c.999G>T	p.Trp253Cys	0.90	0.97	asymptomatic
15		71	M	FGG	7	**c.823G>T**	**p.(Glu275*)**	0.80	1.20	bleeding
17		40	F	FGG		unidenfified		0.80	1.09	bleeding+thrombosis
18		18	M	FGG		unidenfified		1.20	1.10	asymptomatic

Novel variants are in **bold**.
F, Family with at least one relative; *FGB* transcript ID ENST00000302068.9 (NM_005141.5), *FGG* transcript ID ENST00000336098.8 (NM_021870.3); **Digenic variant; [&]Nonsynonymous polymorphism.

In the group of 18 patients with congenital hypofibrinogenemia (CH) and a median age of 28 years (9–71 years), the level of Fbg:coag was 1.02 g/L (0.66–1.45 g/L; IQR: 0.86–1.18 g/L) and Fbg:Ag 1.1 g/L (0.9–1.5 g/L; IQR: 1.02–1.2 g/L). As with CD, 10 (55.5%) of patients were asymptomatic, bleeding and thrombotic events were observed in 6 (33.3%) and 3 (16.6%) of patients, and one of the symptomatic patients had bleeding and thrombotic manifestations. Bleeding was usually provoked and mild in most patients with CD and CH, and no patient suffered from spontaneous or recurrent severe bleeding episodes.

Spectrum of gene variants in Slovak patients with dysfibrinogenemia

In the group of patients with CD comprising 14 unrelated index cases and 4 affected relatives, four different pathogenic variants in a heterozygous form (out of which two had novel variants) were identified in the *FGA* and *FGG* genes. No causal variants were found in the *FGB* gene (Table 4). The most common hot-spot missense pathogenic *FGA* variant c.104G>A p.Arg35His was present in 10 unrelated index cases and two affected relatives. The second hot-spot pathogenic *FGG* variant c.901 C>T p.Arg301Cys was found in two index cases and one relative with diverse clinical phenotypes. These two known hot-spot pathogenic variants represented 83% of variants identified in our patients with CD. One patient was a carrier of a previously unreported frameshift variant in exon 5 of *FGA* c.923_968dup p.(Gly324Lysfs*44). A duplication of 46 nucleotides resulted in a frameshift creating a premature termination codon. A second novel missense variant in exon 8 of *FGG* c.1105C>T p.(His369Tyr) was identified in one index patient and his relative. In silico evaluation of the novel missense variant using MutationTaster and PredictSNP predicted that this variant affects protein function.

In CD patients the bleeding manifestation did not correlate with the type of gene variant or the degree of functional fibrinogen level reduction. Patient No. 16 with a negative history of bleeding bled after having knee arthroscopy despite preoperative fibrinogen replacement. Additional fibrinogen therapy resulted in popliteal vein thrombosis. Patient No. 17 was on continuous warfarin therapy because of recurrent thrombosis of the brachial artery, while his daughter was asymptomatic. In both patients with thrombotic phenotype the concomitant variant c.1433G>A p.Arg478Lys in the *FGB* classified as nonsynonymous polymorphism was present.

Spectrum of gene variants in Slovak patients with hypofibrinogenemia

In a group of 18 patients with CH, eight pathogenic variants were identified, out of which four variants were novel. A variant of uncertain significance in one patient was identified and no causal variant was detected in two patients. All variants were in heterozygous form and located in the *FGB* and *FGG* genes, while none were found in the *FGA* gene (Table 5). The missense variant in exon

7 of *FGB* c.1102T>C predicting p.(Tyr368His), previously described as thrombosis-associated variant Martin II (ref.[30]), was identified in our study in 4 unrelated index cases with different clinical phenotypes. In our group only one woman developed thrombosis during puerperium. In one asymptomatic patient, a missense SNV in exon 4 of *FGB* c.534G>C p.(Lys178Asn) and as yet unreported missense variant in exon 8 of *FGB* c.1427C>T p.(Ser476Leu) were identified. In two brothers (No. 2 and No. 3 from family 1) digenic variants located in *FGB* and *FGG* genes were present, comprising a previously reported alternative splicing pathogenic variant in intron 6 of *FGB* c.959-13_959-10del, resulting into a truncated Bβ chain of 307 residues, known as IVS6-10_16delTTTG, *FGB* IVS6Δ4b (ref.[33]) and the novel SNV in exon 4 *FGG* c.323C>A p.(Ala108Asp). The single nucleotide polymorphism c.1433G>A p.Arg478Lys was also identified in both patients.

Four other pathogenic variants were identified in the *FGG* gene. A missense SNV c.323C>G p.Ala108Gly in exon 4 of *FGG* was present in four unrelated index cases. One patient is a carrier of a missense SNV c.999G>T p.Trp253Cys in exon 7 of *FGG*, previously described as fibrinogen Bratislava. A novel *FGG* variant c.8G>A determining p.(Trp3*) in exon 1 was found in 2 unrelated index cases: one patient with a Fbg:coag level of 0.8 g/L had a bleeding phenotype, while another patient with a similar Fbg:coag level of 0.85 g/L was asymptomatic. A second novel variant, a nonsense variant c.823G>T p.(Glu275*) located in exon 7 of *FGG* was found in one patient with a Fbg:coag level of 0.80 g/L and bleeding symptoms. The bleeding symptoms in patients with CH were mild and mostly provoked (surgery, trauma, delivery). Thrombosis was observed in three patients with CH (Table 5). Patient No. 2, the digenic variants in *FGB* and *FGG* genes, who is also heterozygous for Factor V Leiden, developed cerebral sinus vein thrombosis at the age of 8-years, which was provoked by severe dehydration. Patient No. 7 developed thrombophlebitis of the saphenous vein during puerperium, and patient No. 17, treated with fibrinogen replacement for postpartum haemorrhaging, manifested peripheral deep vein thrombosis.

DISCUSSION

Congenital dysfibrinogenemia and hypofibrinogenemia are classified as rare coagulation disorders. Data on the prevalence of fibrinogen disorders are not accurate as they depend on the different approaches in registration of congenital coagulopathies and different use of routine family studies in different countries. They are also affected by a high proportion of unreported asymptomatic cases as well. The prevalence of CD in European countries is reported to be 15 in 100,000 routinely examined patients[19], and afibrinogenemia 1 in 1,000,000, ranging from 0.7 in 1,000,000 in France to almost 50 in 1,000,000 in countries with a high frequency of consanguinity[20]. The National Registry of Congenital Bleeding Disorders in Slovakia currently lists 447 patients with congenital fibrinogen disorders based on the results of coagulation tests: 302 patients with CD, 144 patients with CH, and 1 patient with afibrinogenemia. The prevalence of registered patients with CD and CH in Slovakia represents 43 and 20 individuals per 10^6 of population, respectively. The diagnosis of fibrinogen disorders is still mostly incidental, either during a routine laboratory examination or before planned surgery. Casini et al. reported that 58% (ref.[7]) of propositi were identified incidentally, similar to 57% (ref.[21]) reported by Shapiro et al.[7,21]. Today, a significant proportion of patients are diagnosed through extended testing within the families of affected individuals, resulting in increasing numbers of patients in the Registry[10].

The clinical phenotype of patients with congenital fibrinogen disorders is very heterogeneous[16,22]. Fibrinogen has several roles in haemostasis: 1) it supports primary haemostasis by participating in the platelets aggregation, 2) forms fibrin clot as the final product of coagulation activation, 3) it has also anticoagulant effect, which resides in the binding of thrombin, thereby inhibiting further action of thrombin in coagulation activation, and finally 4) enables fibrinolysis by binding plasminogen and tissue plasminogen activator into the fibrin network[2]. The bleeding tendency correlates with basal fibrinogen coagulation activity, however, level of at least 0.7 g/L protects from spontaneous bleeding and patients with a level above 1.0 g/L usually do not experience bleeds[23]. However, some patients with CD remain asymptomatic even at very low levels of fibrinogen coagulation activity (Table 4). Low pro-coagulant activity of fibrinogen may be balanced by a simultaneous reduction in its anticoagulant effect, decreased profibrinolytic function or by increased resistance of fibrin network formed by pathological fibrinogen to fibrinolysis[2,9]. In some patients clinical manifestation may be predicted by knowing the gene variant determining the localisation of fibrinogen molecule change and its function impairment (e.g. an association between CD and thrombosis was confirmed in fibrinogen Caracas V, Vlissingen, Melun, Naples, and Dusart)[8]. Initial studies showed that 55% of individuals with CD were asymptomatic, 20% experienced thrombosis and only 25% manifested with bleeding[8]. Shapiro et al. evaluated 35 subjects with CD identified in two UK centres, among whom 57% of the subjects were asymptomatic, 34% experienced bleeding and 9% thrombosis[21]. A previous study evaluating a large group of 75 patients from NHC Bratislava with CD demonstrated asymptomatic, bleeding and thrombotic phenotypes in 53%, 43% and 9% of subjects[10]. In the present study, an asymptomatic course of CD was found in 9 (52.9%), bleeding and thrombosis were observed in 6 (35.3%) and 2 (11.8%) individuals, respectively. Symptoms of bleeding were mild in all but one patient who, despite fibrinogen replacement before knee arthroscopy, experienced major postoperative bleeding requiring further fibrinogen treatment, complicated by popliteal vein thrombosis. Another patient with CD (Table 4, No. 17) suffered from recurrent arterial thrombosis and was treated with warfarin without bleeding complications.

The clinical manifestation of hypofibrinogenemia is also variable, similar to CD. In a previous study from

NHC an asymptomatic course was found in only 38% of patients, while bleeding and thrombosis were present in 44% and 18% of patients, respectively[10]. In the current study, 10 (55.5%) of patients with CH were asymptomatic and 6 (33.3%) and 3 (16.6%) patients experienced bleeding and thrombosis, respectively. The low frequency of bleeding can be explained by the fact that most of our patients with CH had mild hypofibrinogenemia with a median level of 1.02 g/L (IQR 0.86–1.18 g/L). It is known that slightly reduced fibrinogen levels (~1 g/L) are usually sufficient to prevent spontaneous bleeding[23]. The different tendency to bleeding or thrombosis observed in individuals with the same, even very low levels of fibrinogen in either CD or CH, suggests additional confounders that play a role in the clinical phenotype. This is also the reason for the growing interest in the study of genotype-phenotype relationships in congenital fibrinogen disorders. We introduced molecular genetic methods to investigate fibrinogen variants and performed a study aimed at analysing the genetic background in patients with fibrinogen disorders and evaluating the clinical impact of fibrinogen gene variants.

In patients with CD we identified four pathogenic variants, two in *FGA* and two in *FGG* genes. The most prevalent variant within the pathogenic hot-spot of *FGA* c.104G>A p.Arg35His was identified in 10 index patients and 2 relatives accounting for 66.7% of all variants in our cohort with CD. This variant is known as fibrinogen Chapel Hill II, Bicêtre and Bern II. The substitution of arginine for histidine results in delayed or absent FPA cleavage and subsequent prolonged polymerization; however, according to the literature, 65–75% of heterozygous carriers of this variant are asymptomatic[4,19,24]. In our cohort, functional fibrinogen levels ranged between 0.3 and 1.66 g/L. Seven (58.3%) subjects with c.104G>A p.Arg35His were asymptomatic, one even with a fibrinogen level of 0.3 g/L, and five (29.4%) subjects had mild bleeding manifestation. The pathogenic variant c.901C>T p.Arg301Cys at the second hot-spot of *FGG*, known as fibrinogen Osaka II, Morioka or Tokyo II, was present in only 3 patients in our group, with a diverse clinical phenotype in each of them (Table 4). Replacing arginine by cysteine leads to slower fibrin assembly and inaccurate end-to-end alignment of the γ chains, resulting in increased fibre branching[25]. However, as the case of our patient No. 16 has shown, this variant does not protect the patient from thrombosis during fibrinogen replacement therapy.

A novel frameshift variant *FGA* c.923_968dup p.(Gly324Lysfs*44) was identified in one patient. Duplication of 46 nucleotides leads to a frameshift creating a premature termination codon. It could be expected that such structurally different and shorter proteins will be non-functional; however, the functional fibrinogen level in our patient was only slightly reduced (1.53 g/L) and the patient was asymptomatic.

The second novel variant in CD was *FGG* c.1105C>T p.(His369Tyr), identified in the 72-year-old propositus with Fbg:coag/Fbg:Ag levels of 0.4 g/L/2.01 g/L and recurrent arterial thrombosis, and in his 33-year-old asymptomatic daughter with Fbg:coag/Fbg:Ag levels of 0.58 g/L/1.8 g/L. The c.1105C>T p.(His369Tyr) substitution is located in the COOH-terminal part of the γ chain, in which no amino acid substitutions have been reported to date. However, previous studies situate this residue (γHis369) in pocket "a" (localized between residues 363 to 405 of the nascent γ chain) which is crucial for fibrin polymerization[24,26]. The thrombotic manifestation in the propositus could be supported by the presence of another heterozygous variant, *FGB* c.1433G>A p.Arg478Lys[27,28]. Ajjan et al. demonstrated that the substitution c.1433G>A p.Arg478Lys causes significant changes in clot structure, clot rigidity, and fibrinolysis, suggesting an increased risk of atherothrombotic disease[29]. This variant was present in both patients with thrombosis in the CD cohort, also in one of three individuals developing thrombosis in our CH cohort.

In 16 patients from 15 families with hypofibrinogenemia a total of nine different heterozygous variants were identified in the two fibrinogen genes *FGB* and *FGG*. Variant *FGB* c.534G>C p.(Lys178Asn) was detected in an asymptomatic patient. This variant is of uncertain significance (VUS) with conflicting interpretation of pathogenicity. However, Fbg:coag and Fbg:Ag levels were reduced in the woman with this variant (1.1 g/L and 1.4 g/L, respectively).

The pathogenic variant *FGB* c.1102T>C p.(Tyr368His), present in our four unrelated patients, was very recently identified in two individuals with a thrombotic phenotype in the family from northern Slovakia and was designated as fibrinogen Martin II[30]. This variant affects the highly conserved globular C-terminal domain of the fibrinogen β chain causing an impaired hexamer assembly or secretion and results in a reduced concentration of fibrinogen[31]. Of the four patients with c.1102T>C p.(Tyr368His) in our cohort, two patients were bleeding, one was asymptomatic, and one patient developed saphenous vein thrombophlebitis in the puerperium.

A novel missense variant *FGB* c.1427C>T p.(Ser476Leu) was identified in one asymptomatic patient. The substitution of serine for leucine was evaluated by prediction programs (MutationTaster, PredictSNP) as deleterious and therefore was classified as pathogenic. This is due to the substitution of serine as a polar hydrophilic hydroxyl amino acid for leucine with different physical and chemical properties. Leucine is a nonpolar, hydrophobic amino acid usually buried in folded proteins. Also, the Ser476 residue is located in the highly conserved globular C-terminal domain, the integrity of which is essential for the secretion of fibrinogen into the circulation[32].

In the 9-year-old propositus (Table 5, patient No. 2) with thrombosis and his asymptomatic 13-year-old brother (patient No. 3) with CH, we identified an alternative splicing variant c.959-13_959-10del in intron 6 of *FGB*. Four nucleotide deletion is also known as IVS6-10_16delTTTG, FGB IVS6Δ4b and fibrinogen Bizerte, named after its place of discovery in a heterozygous state[33]. This genotype was originally described by Terasawa (variant Matsumoto IX) (ref.[34]). This variant affects the splice acceptor site at the 3' end of intron 6, causing the production of an

aberrant mRNA that includes introns 6 and 7 of the *FGB* gene. The aberrant mRNA is translated into a truncated Bβ chain of 307 residues after an extension of 17 residues followed by a stop codon[33,34]. In both brothers a second novel variant *FGG* c.323C>A p.(Ala108Asp) was also present, inconsistently evaluated by different prediction programs as a polymorphism or as a variant of uncertain significance. This variant affects the same amino acid residue as the variant *FGG* c.323C>G p.Ala108Gly, described as pathogenic. *FGG* c.323C>A p.(Ala108Asp) variant may possibly be pathogenic, but segregation analysis of the symptomatic and asymptomatic family members will be necessary in order to assess the causality of this variant and investigate the cis/trans configuration of the two different variants. The third variant identified in these two individuals was *FGG* c.1433G>A p.Arg478Lys, which was present also in two patients with CD and thrombotic phenotype, suggesting its possible relationship to thrombosis. The nine-year-old propositus with CH, who developed cerebral venous sinus thrombosis, was also heterozygous for Factor V Leiden.

The previously described *FGG* c.323C>G p.Ala108Gly variant (fibrinogen Dunedin) was identified in our study in four unrelated patients. The γAla108 residue is located in the triple helix that separates the E and D domains, where aberrant folding of the helices may explain the decreased fibrinogen levels[35]. Clinical phenotype was asymptomatic in three of four patients.

The pathogenic variant *FGG* c.999G>T p.Trp253Cys identified in one asymptomatic patient was previously described as fibrinogen Bratislava in a patient with CH manifesting spontaneous and provoked bleeding. It is assumed that replacement of tryptophane by the smaller cysteine creates a void leading to local refolding and possibly destabilising normal folding of the γC domain. Extended analysis has revealed normal intracellular assembly of the γ chains but no secretion, confirming the causative nature of the variant[11].

Two novel nonsense variants in the *FGG* gene were identified in our patients with CH. Due to the nature of the variant creating a premature termination codon, resulting in reduced fibrinogen synthesis and low fibrinogen levels, and supported by several prediction programs (MutationTaster, PredictSNP), and by a search engine, aggregator, impact analysis tool for human genetic variation (Varsome), we consider and classify these nonsense variants as pathogenic. The first novel variant *FGG* c.8G>A p.(Trp3*) was detected in 2 unrelated patients, one bleeder and one asymptomatic. This variant was previously identified by the Batorova study group[13] in 7 patients from three Slovak families, but this variant has not yet been reported to the mutation databases. Only two of the 7 carriers of this variant were asymptomatic and 4/7 (57.1%) had mild bleeding symptoms. Two patients suffered from thrombosis, one of whom was also a bleeder, and one woman with concomitant heterozygous Factor V Leiden experienced seven late recurrent foetus losses. In this study only 4/24 (16.7%) operations performed in 7 patients without fibrinogen replacement were complicated by mild to moderate bleeding[13]. The second novel nonsense variant *FGG* c.823G>T p.(Glu275*) was identified in one patient with a bleeding phenotype. In parallel with our finding, Simurda et al. described the same variant in a 45-year-old male from northern Slovakia with CH associated with thrombosis and designated this variant as fibrinogen Martin III (ref.[18]). The γGlu275 residue is a part of the globular C-terminal domain (residues 143–411) which is crucial for fibrinogen assembly and secretion. A premature termination codon (PTC) creates an abnormally truncated γ chain with altered properties and predicts deleteriousness of this variant. Potential deleteriousness was investigated by protein modelling and examining its impact at the transcription level using the Human Splicing Finder web tool which *in silico* predicts to possibly activate a cryptic donor splice site in the exon[18].

We were not able to identify pathogenic variants in two unrelated patients with reduced functional and antigenic fibrinogen levels, one of whom developed thrombosis after fibrinogen replacement for postpartum haemorrhaging (Table 4, No17). This can perhaps be explained by changes outside the *FGA, FGB* and *FGG* coding region, in the promoter region, in the untranslated or non-coding regions. Such changes could not be detected by our approach.

CONCLUSION

The results of this study revealing the six novel pathogenic variants contribute to the mutation spectrum of the fibrinogen genes in the population of patients with fibrinogen disorders in Slovakia and Central Europe as well. We confirmed common hot-spot pathogenic variants to be most prevalent also in our patients with CD. We also demonstrated that a broader spectrum of pathogenic variants is observed in patients with CH, some of which are unique for our population. However, our groups of patients were too small for identification of clear association between genotype and phenotype in these disorders. The existing database of the National Registry of Bleeding Disorders in our country offers a unique opportunity for further molecular-genetic study and evaluation of clinical genotype-phenotype relationships in larger groups of patients with the same causal variant, especially within large families with CD. Our intention, in cooperation with the other centre at the University Hospital in Martin, is to identify the genetic basis in the entire Slovak population with fibrinogen disorders with the aim of contributing to a better understanding of this rare bleeding disorder.

Author contributions: DJar, JCH, AB: designed the research; DJar: performed genetic research; MJ, PD, SD, AV: participated in the research; AB, TP, AK, DJank: performed the clinical part of the study, blood sampling and patients' clinical data collection; DJar, AB: analysed the results, DJar: wrote the paper; AB, JCH, RP, DB: revised the manuscript. All co-authors approved the final version of the manuscript.

Conflicts of interest statement: The authors state that there are no conflicts of interest.

REFERENCES

1. Huang S, Mulvihill ER, Farrell DH, Chung DW, Davie EW. Biosynthesis of human fibrinogen. Subunit interactions and potential intermediates in the assembly. J Biol Chem 1993;268(12):8919-26. doi: 10.1016/S0021-9258(18)52960-4
2. Mosesson MW. Fibrinogen and fibrin structure and functions. J Thromb Haemost 2005;3(8):1894-904. doi: 10.1111/j.1538-7836.2005.01365.x
3. de Maat MP, Verschuur M. Fibrinogen heterogeneity: inherited and noninherited. Curr Opin Hematol 2005;12(5):377-83. doi: 10.1097/01.moh.0000169287.51594.3b
4. Human fibrinogen database presentation, Groupe Français d'étude sur l'Hémostase et la Thrombose. Available from: https://site.geht.org/base-fibrinogene/, 2021 (accessed 1 April 2021).
5. Maghzal GJ, Brennan SO, Homer VM, George PM. The molecular mechanisms of congenital hypofibrinogenaemia. Cell Mol Life Sci 2004;61:1427-38. doi: 10.1007/s00018-004-3458-8
6. Casini A, Undas A, Palla R, Thachil J, de Moerloose P. Subcommittee on Factor XIII and Fibrinogen, Diagnosis and classification of congenital fibrinogen disorders: communication from the SSC of the ISTH. J Thromb Haemost 2018;16(9):1887-90. doi: 10.1111/jth.14216
7. Casini A, Blondon M, Lebreton A, Koegel J, Tintillier V, de Maistre E, Gautier P, Biron C, Neerman-Arbez M, de Moerloose P. Natural history of patients with congenital dysfibrinogenemia. Blood 2015;125(3):553-61. doi: 10.1182/blood-2014-06-582866
8. Haverkate F, Samama M. Familial dysfibrinogenemia and thrombophilia. Report on a study of the SSC Subcommittee on Fibrinogen. Thromb Haemost 1995;73(1):151-61. doi: 10.1055/s-0038-1653741
9. Hanss M, Biot F. A database for human fibrinogen variants. Ann N Y Acad Sci 2001;936:89-90. doi: 10.1111/j.1749-6632.2001.tb03495.x
10. Bátorová A, Jankovičová D, Lazúr J, Prigancová T, de Moerloose P, Neerman-Arbez M, Kušíková M, Mistrík M. Vrodená dysfibrinogenémia a hypofibrinogenémia – molekulová báza a klinický fenotyp. Interná Med 2016;16(3):109-15. (In Slovak)
11. Vu D, de Moerloose P, Batorova A, Lazur J, Palumbo L, Neerman-Arbez M. Hypofibrinogenaemia caused by a novel FGG missense mutation (W253C) in the gamma chain globular domain impairing fibrinogen secretion. J Med Genet 2005;42(9):e57. doi: 10.1136/jmg.2005.033530
12. Bátorová A, Horváthová D, de Moerloose P, Neerman-Arbez M, Mistrík M. Fenotypová a genotypová analýza vrodenej hypofibrinogenémie a dysfibrinogenémie. Vnitř Lék 2005;51(7-8):802-8. (In Slovak)
13. Batorova A, Horvathova D, Mistrik M, de Moerloose P, Neerman-Arbez M. Genetic and Phenotypic Analysis of Families with Inherited Hypo- and Dys-Fibrinogenemia. Fibrinogen Bratislava. Blood 2005;106(11):1794. doi: 10.1182/blood.V106.11.1794.1794
14. Kotlín R, Reicheltová Z, Malý M, Suttnar J, Sobotková A, Salaj P, Hirmerová J, Riedel T, Dyr JE. Two cases of congenital dysfibrinogenemia associated with thrombosis – Fibrinogen Praha III and Fibrinogen Plzeň. Thromb Haemost 2009;102(3):479-86. doi: 10.1160/TH08-11-0771
15. Simurda T, Zolkova J, Kolkova Z, Loderer D, Dobrotova M, Skornova I, Brunclíkova M, Grendar M, Lasabova Z, Stasko J, Kubisz P. Comparison of clinical phenotype with genetic and laboratory results in 31 patients with congenital dysfibrinogenemia in northern Slovakia. Int J Hematol 2020;111(6):795-802. doi: 10.1007/s12185-020-02842-9
16. Wypasek E, Klusowska A, Zdziarska J, Zawilska K, Treliński J, Iwaniec T, Mital A, Pietrys D, Sydor W, Neerman-Arbez M, Undas A. Genetic and clinical characterization of congenital fibrinogen disorders in Polish patients: Identification of three novel fibrinogen gamma chain mutations. Thromb Res 2019;182:133-40. doi: 10.1016/j.thromres.2019.08.012
17. Ceznerova E, Stikarova J, Roselova P, Kaufmanova J, Suttnar J, Louzil J, Kotlin R, Salaj P, Dyr JE. Molecular and Clinical Aspects of Aα Dysfibrinogenemia in 25 Patients from the Czech Republic. Blood 2018;132(Supplement 1):5046. doi: 10.1182/blood-2018-99-118254
18. Simurda T, Caccia S, Asselta R, Zolkova J, Stasko J, Skornova I, Snahnicanova Z, Loderer D, Lasabova Z, Kubisz. Congenital hypofibrinogenemia associated with a novel heterozygous nonsense mutation in the globular C-terminal domain of the γ-chain (p.Glu275Stop). J Thromb Thrombolysis 2020;50(1):233-6. doi: 10.1007/s11239-019-01991-x
19. Shapiro SE. Diagnosis and management of dysfibrinogenemia. Clin Adv Hematol Oncol 2018;16(9):602-5.
20. Casini A, Neerman-Arbez M, de Moerloose P. Heterogeneity of congenital afibrinogenemia, from epidemiology to clinical consequences and management. Blood Rev 2020;26;100793. doi: 10.1016/j.blre.2020.100793
21. Shapiro SE, Phillips E, Manning RA, Morse CV, Murden SL, Laffan MA, Mumford AD. Clinical phenotype, laboratory features and genotype of 35 patients with heritable dysfibrinogenaemia. Br J Haematol 2013;160(2):220-7. doi: 10.1111/bjh.12085
22. Casini A, de Moerloose P, Neerman-Arbez M. Clinical Features and Management of Congenital Fibrinogen Deficiencies. Semin Thromb Hemost 2016;42(4):366-74. doi: 10.1055/s-0036-1571339
23. Peyvandi F, Haertel S, Knaub S, Mannucci PM. Incidence of bleeding symptoms in 100 patients with inherited afibrinogenemia or hypofibrinogenemia. J Thromb Haemost 2006;4(7):1634-7. doi: 10.1111/j.1538-7836.2006.02014.x
24. Zhou J, Ding Q, Chen Y, Ouyang Q, Jiang L, Dai J, Lu Y, Wu X, Liang Q, Wang H, Wang X. Clinical features and molecular basis of 102 Chinese patients with congenital dysfibrinogenemia. Blood Cells, Molecules and Diseases 2015;55(4):308-15. doi: 10.1016/j.bcmd.2015.06.002
25. Mosesson MW, Siebenlist KR, DiOrio JP, Matsuda M, Hainfeld JF, Wall JS. The role of fibrinogen D domain intermolecular association sites in the polymerization of fibrin and fibrinogen Tokyo II (gamma 275 Arg–>Cys). J Clin Invest 1995;96(2):1053-8. doi: 10.1172/JCI118091
26 Shimizu A, Nagel GM, Doolittle RF. Photoaffinity labeling of the primary fibrin polymerization site: isolation and characterization of a labeled cyanogen bromide fragment corresponding to gamma-chain residues 337–379. Proc Natl Acad Sci U S A 1992;89(7):2888-92. doi: 10.1073/pnas.89.7.2888
27. Baumann RE, Henschen AH. Human fibrinogen polymorphic site analysis by restriction endonuclease digestion and allele-specific polymerase chain reaction amplification: identification of polymorphisms at position A alpha 312 and B beta 448. Blood 1993;82:2117-24. doi: 10.1182/blood.V82.7.2117.2117
28. Standeven KF, Grant PJ, Carter AM, Schneider T, Weisel JW, Ariëns RAS. Functional 29Analysis of the Fibrinogen Aα Thr312Ala Polymorphism. Effects on Fibrin Structure and Function. Circulation 2003;107:2326-30. doi: 10.1161/01.CIR.0000066690.89407.CE
29. Ajjan R, Lim BCB, Standeven KF, Harrand R, Dolling S, Phoenix F, Greaves R, Abou-Saleh RH, Connell S, Smith DAM, Weisel JW, Grant PJ, Ariëns RAS. Common variation in the C-terminal region of the fibrinogen β-chain: effects on fibrin structure, fibrinolysis and clot rigidity. Blood 2008;111(2):643-50. doi: 10.1182/blood-2007-05-091231
30. Simurda T, Zolkova J, Snahnicanova Z, Loderer D, Skornova I, Sokol J, Hudecek J, Stasko J, Lasabova Z, Kubisz P. Identification of Two Novel Fibrinogen Bβ Chain Mutations in Two Slovak Families with Quantitative Fibrinogen Disorders. Int J Mol Sci 2018;19(1):100. doi: 10.3390/ijms19010100
31. Casini A, Vilar R, Beauverd Y, Aslan D, Devreese K, Mondelaers V, Alberio L, Gubert C, de Moerloose P, Neerman-Arbez M. Protein modelling to understand FGB mutations leading to congenital hypofibrinogenaemia. Haemophilia 2017;23(4):583-9. doi: 10.1111/hae.13190
32. Neerman-Arbez M, de Moerloose P. Mutations in the fibrinogen gene cluster accounting for congenital afibrinogenemia: an update and report of 10 novel mutations. Hum Mutat 2007;28(6):540-53. doi: 10.1002/humu.20483
33. Amri Y, Dabboubi R, Mghaieth F, Zili M, Messaoud T, Casini A, de Moerloose P, Toumi NEH. Molecular characterization of two hypofibrinogenemic patients associated with a novel FGG IVS6+23T>A substitution and a previously reported FGB IVS6-10_16delTTTG deletion. Haemophilia 2020;26(4):e194-7. doi: 10.1111/hae.13974
34. Terasawa F, Kamijyo Y, Fujihara N, Yamauchi K, Kumagai T, Honda T, Shigematsu S, Okumura N. In vitro transcription of compound heterozygous hypofibrinogenemia Matsumoto IX; first identification of FGB IVS6 deletion of 4 nucleotides and FGG IVS3-2A>G causing abnormal RNA splicing. Clin Chim Acta 2010;411(17-18):1325-9. doi: 10.1016/j.cca.2010.05.030
35. Brennan SO, Fellowes AP, Faed JM, George PM. Hypofibrinogenemia in an individual with 2 coding (gamma82 A-->G and Bbeta235 P-->L) and 2 noncoding mutations. Blood 2000;95(5):1709-13. doi: 10.1182/blood.V95.5.1709.005k04_1709_1713