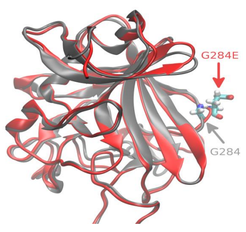
Laboratory Diagnostics of 21 Unrelated Families from Czech Republic with Dysfibrinogenemia and Hypofibrinogenemia



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Introduction

Fibrinogen (FBG) is a key plasmatic glycoprotein of final coagulation phase (2-4.2 g/L) and it plays a crucial role in other physiological processes like platelet aggregation or wound healing.

Quantity and quality of fibrinogen may be affected by inherited disorders, caused by mutations in one of the three genes coding fibrinogen (*FGA, FGB,* and *FGG*).

Congenital dysfibrinogenemia (DYS) is a rare disease characterized by inherited abnormality resulting in functional disorder of fibrinogen molecule. Congenital hypofibrinogenemia (HYPO) is a defect in total fibrinogen concentration caused by impaired synthesis, assemble or expression of fibrinogen out of hepatocytes.

In this work, we evaluated 21 unrelated families, altogether 36 patients, with mutation in fibrinogen found in the Czech population.

Tab.1 List of congenital mutations of fibrinogen

GENE	MUTATION	FAMILY	FBG Clauss [g/L]	FBG TOTAL [g/L]	PHENO TYPE	DYS/ HYPO
FGA	Aα Gly13Glu	1	0.7	3.5	T	DYS
			0.6	3.0	A	
		2	0.8	4.3	A	
		3	0.5	3.2	B	
			0.9	2.3	A	
		4	0.9	3.4	A	
			0.7	3.2	A	
			0.7	3.1	A	
		5	0.4	3.3	B	
			0.5	2.8	A	
			0.8	2.9	A	
		6	0.6	3.5	A	
	Aα Arg16Cys	7	0.5	2.3	B	
		8	0.5	3.3	B	
			N/A	N/A	B	
			N/A	N/A	A	
	Aα Arg16His	9	0.6	3.3	B	
		10	0.6	N/A	A	
			0.5	1.8	A	
			0.6	2.0	A	
			0.8	N/A	A	
		11	0.9	3.6	A	
		12	0.9	2.6	T	
	Aα Arg19Gly	13	0.9	3.9	B	
			1.0	2.3	A	
			1.0	N/A	A	
FGB	Bβ Gly414Ser	14	1.6	1.9	A	HYPO
	Bβ Tyr416Stop	15	1.3	1.7	A	
FGG	γ Trp3Stop	16	1.7	1.7	T	
			N/A	N/A	A	
	γ Thr34Ala	17	2.3	2.0	B	
	γ Ala82Gly	18	1.6	1.9	B	
			1.5	N/A	A	
		19	1.9	2.0	A	
	γ Tyr211His	20	1.3	1.8	A	
	γ Gly284Glu	21	1.2	1.8	A	

(N/A – not available, A -asymptomatic, B - bleeding, T - thrombosis)

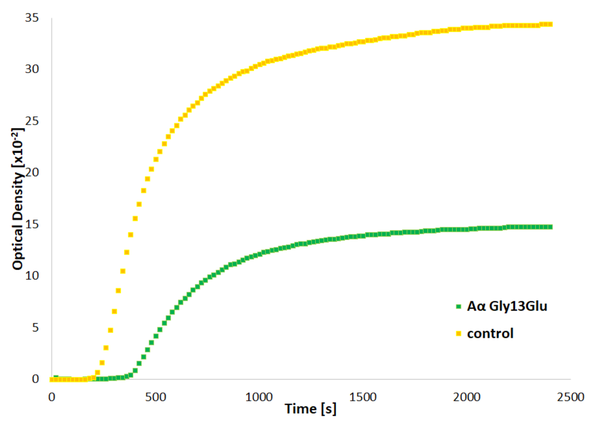

Fig.1 Polymerization curve of plasma sample of patient with Aα Gly13Glu induced by thrombin 0.5 U/mL

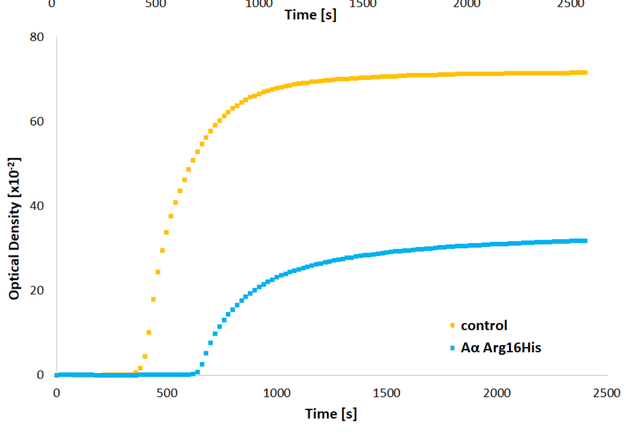

Fig.2 Polymerization curve of plasma sample of patient with Aα Arg16His induced by reptilase

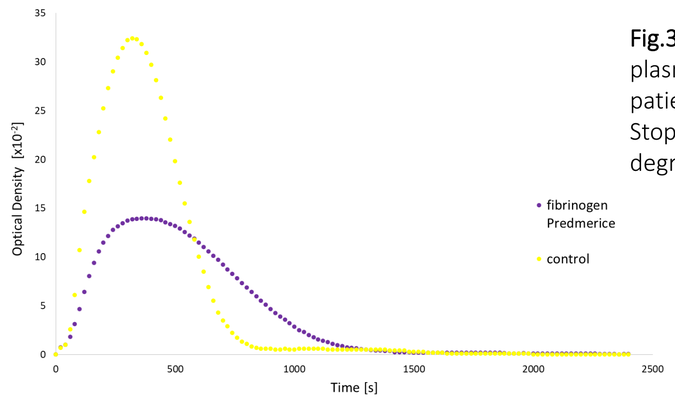

Fig.3 Fibrinolysis of plasma sample of patient with γ Trp3 Stop with prolonged degradation phase

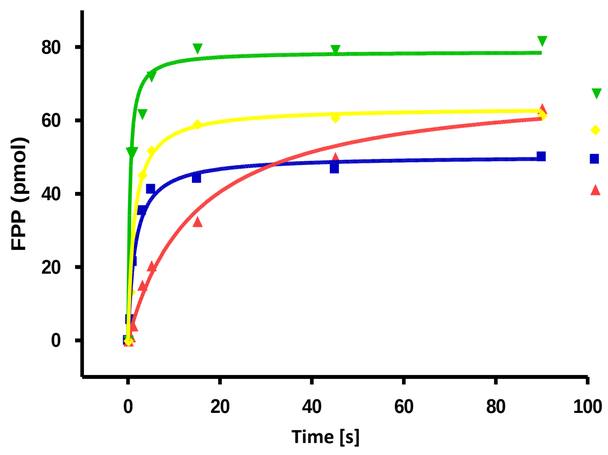

Fig.4 Kinetics of FPP release of patient with Aα Arg16Cys and healthy control initiated by thrombin. Measured by RP-HPLC with 200 nm UV detection.

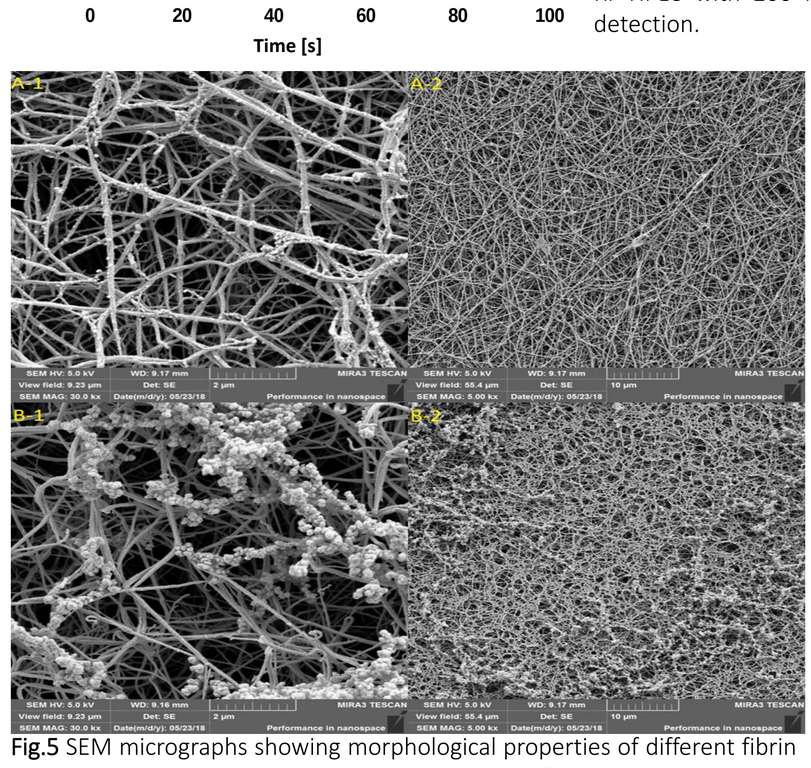

Fig.5 SEM micrographs showing morphological properties of different fibrin fibres of healthy control (A) and patient with γ Trp3Stop (B) at 30.0 kx magnification (A-1, B-1) and 5.00 kx magnification (A-2, B-2)

Conclusion

To conclude, here, we have reported **36 cases of congenital fibrinogen** disorders. Patients diagnosed with dysfibrinogenemia (27 cases) mostly manifested asymptomatically (18 cases), 7 had bleeding and 2 thrombotic phenotype. Hypofibrinogenemia was classified in 9 cases, 3 of them were caused by novel mutation.

Laboratory diagnostics of plasma samples showed a connection between the mutations and clinical manifestations of these patients. Therefore, our observations support the variability of clinical phenotypes caused by wide spectrum of mutations and provide a better understanding of the behavior and role of inherited abnormal fibrinogen in blood coagulation.

Methods

All patients with suspected congenital fibrinogen disorder, based on pathological coagulation test results or clinical manifestation, were laboratory examined by these methods:

1) **PCR and Sanger sequencing** – to identify mutations in DNA
2) **Fibrin polymerization curves and Fibrinolysis** – functional testing of the impact of mutation on the correct properties of FBG
3) **Quantification of Released Fibrinopeptides (FPP) by RP-HPLC** – to evaluate the kinetics of hydrolytic cleavage of FPP by thrombin
4) **Confocal Laser Microscopy and/or Scanning Electron Microscopy** – to study morphological properties of fibrin net formed by mutated FBG

More specialized methods used in some cases:

5) **Molecular Dynamics Simulations** – to analyze physical movements of molecules and atoms in location of mutation
6) **Tandem Mass Spectrometry** – to verify the classification of disorder based on the detection of mutated amino acid in tryptic digested peptide
7) **Thrombelastography** – to study viscoelastic properties of FBG

Results

Here, we evaluated **36 patients** (Tab. 1), belonging to **21 unrelated families**, with heterozygous missense mutations.

Dysfibrinogenemia was diagnosed in 27 cases, mostly in exon 2 of FGA gene, around thrombin cleavage site in N-termini of Aα chain. This place is essential for proper cleavage of FPP and further fibrin polymerization therefore patients have a prolonged coagulation tests, impaired fibrin polymerization curves (Fig. 1 and 2) and decreased amounts of released FPP (Fig. 4)

Hypofibrinogenemia was found in 9 remaining cases, moreover, we identified 3 novel mutations (**γ Trp3Stop, γ Thr34Ala** and **Bβ Tyr416Stop**). Laboratory tests confirmed an decreased amount of total fibrinogen in circulation. More interestingly, mutation γ Trp3Stop showed an impaired fibrinolytic degradation phase (Fig. 3) and abnormal structure of fibrin clot (Fig. 5).

Clinical features of patients manifested asymptomatically in 24 cases, 9 cases were associated with bleeding and 3 cases with thrombosis (Tab. 1).

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