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, assembly 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.

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 heterogeneous missense mutations.

Dysfibrinogenemia was diagnosed in 27 cases, mostly in exon 2 of FGA gene, around thrombin cleavage site in N-terminus 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 (y Trp3Stop, y Thr34Ala and B8 Tyr416Stop). Laboratory tests confirmed an increased amount of total fibrinogen in circulation. More interestingly, mutation y Trp3Stop caused an impaired fibrinolytic degradation phase (Fig. 3) and abnormal structure of fibrin clot (Fig. 5).

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

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Fig. 1 Polymerization curve of plasma sample of patient with Aα Gly835Trp induced by thrombin 0.5 U/mL.

Fig. 2 Polymerization curve of plasma sample of patient with Aα Arg16Cys induced by reptilase.

Fig. 3 Fibronectin analysis of plasma sample of patient with y Trp3Stop 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 y Trp3Stop (B) at 30.0 kx magnification (A-1, B-1) and 5.0 kx magnification (A-2, B-2)