Dysfibrinogenaemia associated with a novel heterozygous mutation in FGB (c.680delG) and a mild clinical history of bleeding

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Fibrinogen is a hexameric protein composed of \(\alpha\), \(\beta\) and \(\gamma\) chain pairs [1] encoded by three paralogous genes, \(FGA\), \(FGB\) and \(FGG\) [2]. Fibrinogen variants are the consequence of mutations in \(FGA\), \(FGB\) or \(FGG\) and may result in afibrinogenaemia, hypofibrinogenaemia, dysfibrinogenaemia or hypodysfibrinogenaemia. Dysfibrinogenaemia is characterized by a fibrinogen structural abnormality responsible for altered functional properties [3]. The majority of the mutations are located in \(FGA\), followed by \(FGG\) and the fewest in \(FGB\) [4]. Mutations in \(FGB\) are functionally interesting because \(\beta\) chain synthesis is thought to be rate-limiting for mature fibrinogen production [5]. The associated clinical symptoms range from easy bruising, excessive bleeding (i.e. nose and mucosal bleeds, genitourinary tract, gastrointestinal tract and intracranial haemorrhage) to thromboembolic events. A database for human fibrinogen variants is available at http://www.geht.org.

Here we report a patient with dysfibrinogenaemia. This is a 49-year-old Swiss female referred in September 2010 to Lausanne University Hospital for investigation of a bleeding disorder. She presented with a history of post-partum bleeding after the vaginal delivery of her first child in 1987 without blood transfusion. Her two following children were born by vaginal delivery without complication. In 2006, she experienced massive bleeding requiring blood transfusions after a hysterectomy for a fibromyoma and in 2010 local bleeding after a fine needle aspiration to investigate a breast lump for which no blood transfusion was required. The patient complained about menorrhagia until hysterectomy and required iron replacement therapy for iron deficiency anemia. Tooth extractions were performed without excessive bleeding. She did not have epistaxis, easy bruising, spontaneous haematomas or gastrointestinal bleeding. As in Shapiro et al. publication [6], we used the International Society on Thrombosis and Hemostasis concensus bleeding assessment tool, and her score was 2. She had no history of thrombosis or miscarriage. Her family history was uneventful with the exception of her daughter who suffers from menorrhagia.

Blood count and morphology, activated partial thromboplastin, prothrombin and thrombin times, factor VIII, von Willebrand factor antigen and activity and factor XIII were normal. Platelet Function Analyzer-100 (PFA-100) closure time (collagen/ADP and collagen/epinephrine cartridges) was within the normal range. Platelet aggregation measurement was not performed.

Functional fibrinogen level (Clauss method) was decreased to 1.4 g/l (\(N = 2.0–4.0\)). Immunoreactive fibrinogen level was 2.3 g/l (\(N = 1.5–3.5\)), implying a qualitative defect of fibrinogen. Family screening showed that her daughter had a functional fibrinogen level of 2.4 g/l. Her two sons were asymptomatic and refused further investigations.

After obtaining patient-informed consent, blood was collected for genetic analysis by amplification and sequencing of all the coding sequences and the splicing sites of the \(FGA\), \(FGB\) and \(FGG\) genes. We identified a novel heterozygous deletion in \(FGB\) exon 4: c.680delG. This single base-pair deletion leads to a frame shift and is predicted to produce an aberrant fibrinogen beta-chain with 45 abnormal residues (starting with Cys227Ser, numbered from the initiator Met) before a premature stop codon TGA. Such a severe truncating mutation, eliminating the entire beta-chain C-terminal globular domain, which is important for fibrinogen secretion [7], may intuitively be thought more likely to be associated with a quantitative rather than a qualitative defect. However, beta-chains deleted from residue 238, which have an intact coiled-coil domain (residues 157–222), have previously been shown to be assembled and secreted [8]. It is therefore likely that the aberrant beta-chain produced as a consequence of c.608delG, which also preserves an intact coiled-coil, is present in the patient’s circulation in the form of abnormal hexamers, which impair normal fibrin clot formation, thus causing the observed bleeding symptoms.

In the database for human fibrinogen variants (http://www.geht.org), version 37, updated January 2012), 84 \(FGB\) variants (59 distinct mutations) are reported accounting for 39 dysfibrinogenaemic (d), 21 afibrinogenaemic (a), 22 hypofibrinogenaemic (h) and two hypodysfibrinogenaemic cases (hd). Thirty-two cases presented with haemorrhage (10d, 9a, 11 h, 2hd), 15 with thrombosis (14d, 1a), six with both complications (4a, 2h), 15 were reported asymptomatic (10d, 5h) and for 15 cases clinical data were missing (4d, 7a, 4h). To our knowledge, there is only one case reported with a variant affecting the same amino acid residue as our patient [9]. This is an afibrinogenaemic patient homozygous for a missense mutation (\(FGB\) c.680G > T, Cys227Phe) who showed spontaneous intracerebral bleeding. Cys227 is located in the conserved fibrinogen beta globular C-ter domain and is involved in the formation of a disulfide bridge with Cys161 in the gamma-chain. In this case, the complete fibrinogen
deficiency can be explained by an abnormal three-dimensional structure of the beta-chain C-ter domain, which does not allow hexamer assembly and secretion as previously mentioned [7].

Our patient, a heterozygous carrier of 680delG with dysfibrinogenaemia, had an abnormal bleeding score. Her first bleeding event was a postpartum haemorrhage after a difficult vaginal delivery, a situation also occurring in normal subjects. Moreover, it is worth noticing that her two following vaginal deliveries were not complicated by bleeding. Referring to the literature [10], women with dysfibrinogenaemia and a history of recurrent poor pregnancy outcome were treated with prophylactic fibrinogen concentrate infusions as soon as pregnancy was confirmed using a target trough fibrinogen level of 1 g/l. In afibrinogenaemic/hypofibrinogenaemic patients, fibrinogen levels are maintained over 1 g/l during pregnancy and for delivery. According to these guidelines, our patient would not have received a prophylactic substitution during her first pregnancy and delivery. Indeed, her baseline level was above 1 g/l, so no further measures would have been undertaken. Knowing the diagnosis, when the postpartum haemorrhage occurred, a substitution might have achieved an earlier haemostatic efficacy and thereby a reduced overall bleeding.

Both bleeding after hysterectomy and local bleeding after a fine needle aspiration in a breast lump can be considered standard complications after such procedures.

According to the UK guidelines [11], patients with dysfibrinogenaemia with a bleeding phenotype should be treated with fibrinogen concentrate preoperatively to raise and maintain the fibrinogen level to 1 g/l above their baseline level until haemostasis is secure and 0.5 g/l until wound healing is complete. Knowing the diagnosis, our patient would have been prophylactically substituted, which could have prevented the bleeding.

In conclusion, we have identified a novel heterozygous substitution, which could have prevented the bleeding. Following the patient’s diagnosis, our patient would have been prophylactically treated with fibrinogen concentrate preoperatively to raise and maintain the fibrinogen level to 1 g/l above their baseline level until haemostasis is secure and 0.5 g/l until wound healing is complete. Knowing the diagnosis, our patient would have been prophylactically substituted, which could have prevented the bleeding. In conclusion, we have identified a novel heterozygous substitution, which could have prevented the bleeding. Following the patient’s diagnosis, our patient would have been prophylactically treated with fibrinogen concentrate preoperatively to raise and maintain the fibrinogen level to 1 g/l above their baseline level until haemostasis is secure and 0.5 g/l until wound healing is complete. Knowing the diagnosis, our patient would have been prophylactically substituted, which could have prevented the bleeding.