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LETTER TO THE EDITOR

Splice site mutation in factor X gene manifesting as severe intracranial haemorrhage in neonatal period with a challenging treatment course

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Factor X (FX) deficiency is a rare autosomal recessive disorder with an estimated incidence of 1:1 000 000. However, its incidence is almost 8–10-fold higher among populations where consanguinity is common. The prevalence of heterozygous individuals can be difficult to calculate, as many of these patients are asymptomatic but it has been estimated to be around one in 500 [1]. Patients with homozygous FX deficiency most commonly present with mucocutaneous bleeding symptoms, but musculoskeletal, intracranial, gastrointestinal or genitourinary bleeding can also occur. Intracranial bleeds have been seen in up to 15% of patients with the majority of them occurring in patients with severe FX deficiency (activity <1%). The half-life of FX in adults is about 48 h, while infused plasma products have a shorter half-life of about 20–40 h [2]. In the United States, traditionally fresh frozen plasma and prothrombin complex concentrate have been utilized for the treatment and prevention of bleeding episodes. A purified FX product has recently been approved by the FDA in September 2015, but only for adults and children over 12 years of age. Given the scarcity of data with regard to variability in factor half-life and dosing guidelines especially in the neonatal age group, management of severe bleeds can be difficult. We describe our successful management of a case of severe FX deficiency due to a splice site single base pair substitution presenting in neonatal period with an intracranial haemorrhage. Initial treatment was challenging due to short factor half-life.

A full-term female infant, born via normal spontaneous vaginal delivery to parents of Southeast Asian origin and consanguineous marriage presented on day 2 of life with melena and generalized tonic–clonic seizures. She was found to have anaemia (Hb 5 g dL^{-1}) and thrombocytopenia (platelets 80 000 μL^{-1}). Coagulation studies revealed an INR of 9.4, with a PTT of >200 s and a PT of >100 s. All coagulation factor levels were within the normal range for newborns except for FX activity, which was undetectable, consistent with a diagnosis of severe FX deficiency. Head ultrasound showed a significant left parieto-occipital haemorrhage. There was no family history of bleeding disorders. The father and mother's FX levels were 67% and 86% respectively. After initial empirical treatment with twice daily fresh frozen plasma, in the absence of a purified FX product, we elected to use Prothrombin Complex Concentrate, Bebulin™ (140 U of factor X for every 100 U of factor IX), as providing the best available ratio of FX [2]. Based upon our review of literature on use of PCC products in neonates with FX deficiency, doses ranged from 20–70 U kg^{-1} [2–5]. We initially started therapy at 30 U kg^{-1}. One hour peak FX level after the first dose was 39%, but the trough decreased to 11% at 12 h after the dose. The Bebulin™ dose was escalated to 40 U kg^{-1} but 24-h trough levels remained lower than anticipated at 10–11% with a maximum of 16–18% (Fig. 1). Mixing studies did not reveal the presence of an inhibitor. She clinically remained stable and exhibited no further signs of bleeding on a dose of 40 U kg^{-1} despite the low troughs. Since Bebulin™, in addition to factors X and IX also contains significant amounts of factor II (120 U of FII for every 100 U of FX) there was concerns about a possible prothrombotic risk associated with higher dosing, especially in a neonate, in whom the natural tendency is towards a more prothrombotic state. Hence, the decision was made to continue treatment at 40 U kg^{-1}. Treatment was continued for 14 days under close monitoring. No further bleeding symptoms were observed and she improved clinically. Upon discharge, she was successfully maintained on prophylaxis with Bebulin 75 U kg^{-1}, twice a week, with preinfusion trough levels between 1% and 5%.

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In order to identify the mutation responsible for the patient's FX deficiency, genomic DNA was isolated from fresh peripheral blood using the Gentra Puregene Blood Kit (Qiagen). Primers were designed for all eight exons of the FX gene including the flanking intronic regions. Patient, parental and normal reference samples were amplified by PCR and products were visualized on 1% agarose with ethidium bromide. PCR amplicons were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced using Sanger sequencing. Analyses using the Mutation Surveyor program (Softgenetics) revealed a homozygous single base pair substitution within the first intron (IVS1-1G>C) in the patient's DNA when compared with the FX reference genome (NM_000504.3). Heterozygous mutations at the same location in the parental samples were also identified, confirming an autosomal recessive inheritance pattern (Fig. 2). Primers utilized for this region were: Exon 2 Forward-ATTCCCTTGGCAGAGAGGAC and Exon 2 Reverse-CTGTGGCCTGAGCTCCTTAC.

FX is a vitamin K-dependent serine protease that plays an essential role in the coagulation cascade. As the first enzyme of the final common coagulation pathway, FX is crucial for both the contact activation (intrinsic) and tissue factor (extrinsic) pathways. FX's physiologic importance is underlined by the potentially severe and lethal consequences of its absence, as a total deficiency of FX has been shown to cause partial embryonic lethality [6].

Replacement of FX can be problematic, especially in the neonatal age group, where data regarding dosing and metabolism is scarce. The recently FDA-approved FX concentrate, Coagadex™, is currently only approved for children older than 12 years. Thus currently, neonatal replacement can be achieved by use of fresh frozen plasma or prothrombin complex concentrates (PCC). Those PCC products which provide a 1:1 ratio of Factor X: IX, are reportedly expected to raise factor levels by 1.5% for every 1 U kg^{-1} infused. Bebulin™ has a higher ratio (1.4:1) of FX to FIX; thus, we would expect a proportionally higher rise for every 1 U kg^{-1} of Bebulin. In adults, the reported half-life of infused FX ranges from 20 to 40 h. In our patient, despite aggressive factor replacement therapy at 40 U kg^{-1} daily, 24-h trough levels remained low at 10–18% (Fig. 1), as detailed above, suggesting a more rapid clearance. While some previous cases in the literature reported half-lives consistent with the adult range, two other reports revealed half-lives less than 20 h in young infants [3,7]. Additional cases reporting fatal bleeding episodes in children despite

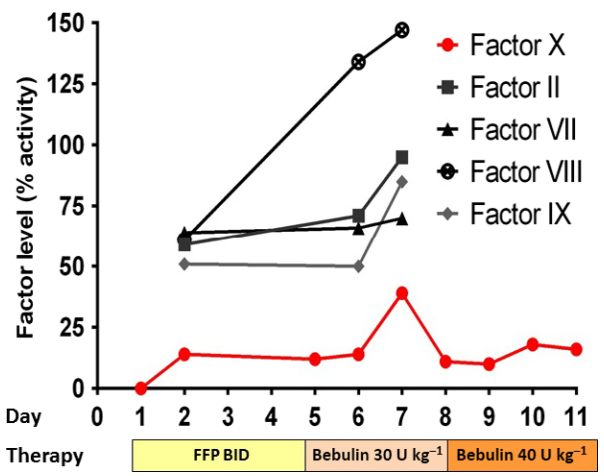

Fig. 1. Factor levels after treatment: Patient was initially treated empirically with twice daily FFP and transitioned to Bebulin™ at a starting dose of 30 U kg^{-1} on day 5. Though the 1 h post-Bebulin™ peak was encouraging at 39%, 12-h troughs remained low at 12–18% even at higher doses of 40 U kg^{-1}. Other factors contained in Bebulin™ (FII, VII, IX) showed appropriate rise with treatment. Factor VIII levels were monitored for prothrombotic potential and were stable between 130% and 150%. All levels shown are trough levels drawn 12 h post dosing, except the levels on day 7, which were drawn 1 h after the Bebulin™. [Colour figure can be viewed at wileyonlinelibrary.com]

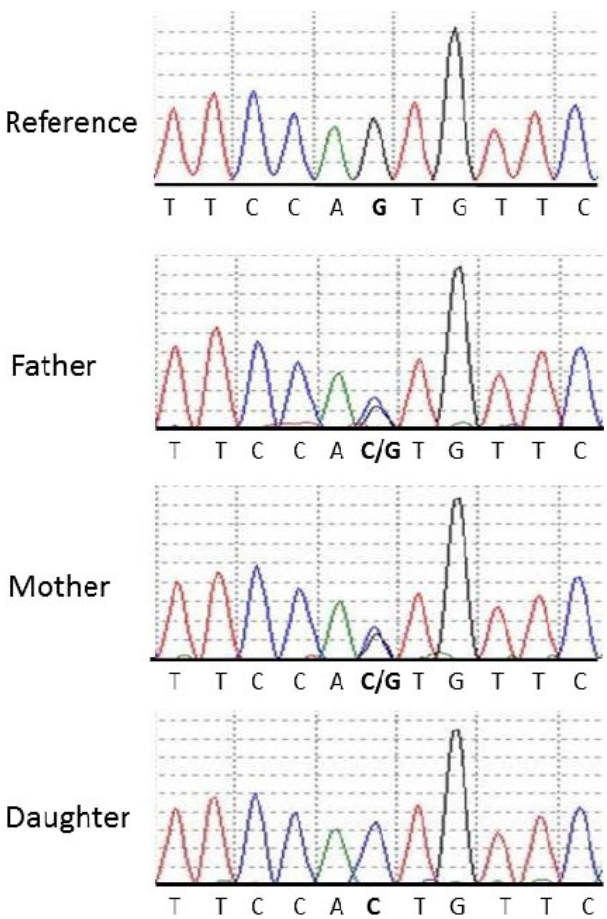

Fig. 2. Sanger sequencing traces. Analyses revealed a homozygous single base pair substitution within the first intron (IVS1-1G>C) in the patient's DNA when compared with the F10 reference genome (NM_000504.3). Heterozygous mutations (C/G) at the same location in the parental samples were also identified, confirming an autosomal recessive inheritance pattern. [Colour figure can be viewed at wileyonlinelibrary.com]

standard prophylaxis based on adult patterns, may also indicate a more rapid half-life in these patients [8]. While we were unable to calculate a precise half-life for our patient due to difficulties with venous access precluding collection of a sufficient number of time points, a rough estimate based upon the drop from the peak to trough levels, would suggest that her half-life was significantly shorter than published reports.

To date, 146 FX mutations have been identified (The Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/gene.php?gene=F10), but a consistent correlation between the genotype and phenotype of these mutations has not been observed [9,10]. The IVS1-1G>C mutation, which was previously reported by Millar *et al.*, results in a 13 bp deletion in exon 2 and defective propeptide cleavage and processing [9]. It was reported as having a severe phenotype, but no specific mention of intracranial haemorrhage was made. This mutation was also not included in a review of FX mutations and risk for intracranial haemorrhage by Raush *et al.*[4]. Thus, this is the first report of the IVS1-1G>C mutation in FX deficiency presenting with intracranial haemorrhage at birth. Our case would suggest that this mutation is a high risk one and patients with this mutation should be considered for prophylactic treatment with FX.

Our patient is now 2.5 years old. Initially as outpatient, she was maintained on prophylaxis with Bebulin at 100 U kg^{-1} twice a week and then gradually weaned to 120 U kg^{-1} once a week. Trough levels have fluctuated between 1% and 5% without any further bleeding symptoms. She has been growing well, with only mild speech and fine motor delays. Our management strategy during her acute presentation in the neonatal stage was successful in preventing further bleeding and no episodes of thrombosis were observed despite higher dosing of Bebulin, suggesting that this is a viable treatment option for neonates with FX deficiency in the absence of a purified FX product or in resource-limited settings. Our case also adds to a body of literature supporting the theory that the metabolism of coagulation factors in neonates is different from that of adults or older children, calling for close monitoring of half-lives and individualized treatment plans for every patient [3,7,8]. To the best of our knowledge, the off label use of the purified FX product (Coagadex ™) has not been reported in neonates.

Rare coagulation disorders can present significant treatment challenges, especially when they present in the neonatal period with severe manifestations. As demonstrated in our patient, coagulation factor metabolism in neonates is different from those in older children and adults, necessitating higher and/or more frequent dosing. Larger multicenter studies are necessary for the development of evidence-based guidelines for management of such rare but potentially life-threatening disorders.

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Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

Author Contributions

P.M and T.B were involved in the conceptualization and design of the manuscript, P.M and J.C collected and organized patient clinical data, M.N supervised the laboratory diagnosis and testing for the patient, E.R and W.LC provided critical review and analysis of the data, T.B, C.L.J, B.L and J.M were involved in the molecular diagnosis and sequencing of patient and parents. All authors read and agreed to the data presented in the manuscript.

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