Identification of a novel factor X deletion in combination with a missense mutation in the F10 gene

Genotype-phenotype correlation in a girl with severe factor X deficiency

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Summary
The genotype-phenotype relationship of compound heterozygous factor X deficiency in a young girl with severe factor X deficiency and bleeding symptoms is characterized. We identified a novel deletion of exon 6 and a missense mutation (c.856G>A, Val286Met) in exon 7 of the F10 gene leading to a compound heterozygous state and causing severe factor X deficiency. Therapeutic options for patients with symptomatic factor X deficiency are demonstrated.

Factor X is a vitamin K dependent plasma glycoprotein that plays a central role for both the intrinsic and the extrinsic part of the coagulation cascade. Factor Xa is composed of a 17 kDa light chain and a 45 kDa heavy chain associated through a disulfide bond (6). The heavy chain contains the catalytic serine protease domain that is structurally homologous to that of other serine proteases (3). The human F10 gene is located on chromosome 13q34. It is composed of eight exons spanning a region of 25 kb.

Congenital factor X deficiency is one of the rarest autosomal-recessive bleeding disorders. The incidence of homozygous factor X deficiency is 1:1 000 000 in the general population. There are only very few reports of compound heterozygous inheritance of severe factor X deficiency. Factor X levels do not correlate well with the clinical phenotype and the severity of the haemorrhagic diathesis. Sometimes symptoms do not emerge until adolescence, and heterozygotes are often asymptomatic.

Modern classification of factor X variants is based on the molecular level, and multiple mutations and deletions have been identified (1). At present, no conclusive data on genotype-phenotype relation are available.

There is no purified factor X concentrate available, so far, so that therapeutic options are limited: Application of red blood cells, fresh frozen plasma (FFP), and intermediate-purity factor IX concentrates (containing factor IX and factor X) has been described.

A girl with FX deficiency

We report on a young girl with severe factor X deficiency who first presented to our hospital at the age of 15 months. The blood tests which had been performed because of a serious otitis media had shown a severe microcytic anaemia (haemoglobin 5.1 g/dl) and pathologic values for the global coagulation tests (PT 9%, INR 9.67, aPTT 125 s). Factor X-activity (FX:C) was analysed using a one-stage method: Factor X deficient plasma from Technoclone® with a thromboplastin reagent (Thromborel® S from Dade Behring). Analysis of the clotting factors revealed that FX:C was less than 1% corresponding to severe factor X deficiency.

At that time the patient had experienced neither any major haematomas nor epistaxis nor any other bleeding episode. The anaemia turned out to be in part diet-related.

One year after the first admission she presented with a tooth bleeding after a trauma when she had fallen on the face. The bleeding could be stopped initially by intravenous injection of tranexamic acid and subsequent application of a plasma-derived factor-X-containing factor IX preparation. After 25 U/kg body weight of this intermediate-purity...
factor IX concentrate the plasmatic FX : C increased from <1% to 74%, PT from 7% to 86%, and the aPTT shortened from 147 s to 46 s. Four teeth were successfully removed under this treatment.

Eight months later the girl was admitted with haemarthros of the left ankle after a minor trauma (Fig. 1). She recovered and was free of pain after 29 U/kg body weight of the factor-X-containing factor IX preparation and could be discharged the next day.

Another four months later she presented with a mucosal bleeding due to a stomatitis aphthosa. After injection of 40 U/kg body weight of the intermediate-purity factor IX concentrate the bleeding from the mucosal lesions stopped. The child received another 40 U/kg body weight and the lesions healed very fast.

During the following three years the patient was recurrently readmitted because of haematomas of the right sole of foot, an assumed joint bleeding of the right knee, a large haemorrhage at the left calf and several minor mucosal bleeding episodes. The patient always responded clinically very well to substitution with human intermediate-purity factor IX concentrate (containing also factor X) without any side effects. She never experienced life-threatening events such as intracranial haemorrhage.

DNA analysis of the F10 gene demonstrated a compound heterozygous state: The patient was heterozygous for a large deletion of exon 6 of the F10 gene which has not been described in the literature or databases, so far. She was also heterozygous for the missense mutation c.856G>A, Val286Met in exon 7 that codes for the catalytic region of factor X (nomenclature according to the Human Genome Variation Society: HGVS at www.genomic.unimelb.edu.au/mdl/mutnomen/). The mother turned out to be heterozygous for the deletion and the father for the above mentioned missense mutation.

### Discussion

We identified a compound heterozygous state of the F10 gene resulting in severe factor X deficiency (FX:C<1%) in a young girl with severe bleeding symptoms. The clinical presentation of factor X deficiency is variable and is mainly characterized by soft tissue and mucosal bleeding. In older children and teenagers episodes of epistaxis and menorrhagia have been described. A haemophilia-like bleeding pattern with haemarthros and intramuscular haemorrhage is very rare. Young children with factor X deficiency are often less symptomatic and usually do not show severe bleeding episodes. Our young patient showed severe clinical haemorrhagic symptoms which is most probably caused by the very low FX:C.

Most affected individuals with severe factor X deficiency have low but measurable levels of FX : C (4). Factor X knockout mice die in utero or shortly after birth suggesting that complete absence of FX in plasma may be incompatible with life in mice (2). Interestingly, in this patient FX:C was less than 1%.

Normally, mature FX is activated by the cleavage of the activation peptide by factor IXa (in the intrinsic pathway), or by factor VIIa (in the extrinsic pathway). Factor Xa then converts prothrombin to thrombin in the presence of factor Va, Ca$^{2+}$ and phospholipids. Interestingly, in our patient both aPTT (the intrinsic) and PT (the extrinsic pathway of the coagulation cascade) were severely affected.

This is the first characterization of a patient with severe factor X deficiency caused by a deletion of the whole exon 6 and by a concomitant missense mutation of the F10 gene.

Deletions of single exons causing factor X deficiency are very uncommon since most of the reported genetic defects in the F10 gene are missense mutations. A deletion of exon 6 of the F10 gene has not been described in the literature, so far. The deletion that was identified in our patient is exceptionally large (at least 245 bp). Exon 6 codes for the activation peptide of factor X. Former analyses of mutations within the activation peptide showed a markedly reduced FX: C. Additionally, defects within the activation peptide seem to lead to an increased plasma clearance rate of factor X (6).

The reported clinical phenotypes of patients with alterations in the activation peptide on the molecular level are heterogeneous and range from mild to severe bleeding diatheses.

The patient’s mother is heterozygous for this deletion and exhibits a slightly diminished FX : C of 56% without increased bleeding symptoms. The mother has normal values for PT but shows a prolonged aPTT (50 s).

Approximately 75% of patients with factor X deficiency have causative missense mutations (4). The heterozygous missense mutation c.856G>A, Val286Met found in our patient locates to the end of exon 7 in the catalytic domain of factor X. This mutation has not been published in the international factor X mutation register, so far. Extensive literature analysis revealed that the mutation c.856G>A, Val286Met is identical with the mutation found in the first index patient: Mr. Stuart (6).

Mutations in the catalytic domain usually go along with a low FX : C and a severe bleeding tendency in the homozygous state. This mutation was inherited from the paternal side, and the asymptomatic heterozygous father shows reduced FX : C of 33%, slightly prolonged aPTT and a borderline PT of 70%.

The combination of these two genetic defects in our case offers interesting insights into the genotype-phenotype correlation in patients with the rare constellation of compound heterozygous factor X deficiency. In a former study 7% of subjects with causative mutations in the F10 gene were compound heterozygous (2). All of them were asymptomatic and showed a severe bleeding pattern that was comparable to homozygous patients. The case highlights that in a patient with a compound heterozygous state causing severe factor X deficiency, a severe course of disease can develop.
Conclusion

Molecular genetic analysis and further genotype-phenotype correlation of patients with factor X deficiency is needed to comprehend the bleeding tendency and to specify the optimal treatment. In our case, substitution with a plasma-derived factor-X-containing factor IX preparation was an effective therapy option in the event of a severe bleeding.

References