Rare coagulation disorders

chapter 6

Summary

Clinical symptoms. The type of clinical manifestations and optimal treatment are not well established for recessively bleeding disorders, that have prevalences in the general population varying between 1:500,000 (factor VII deficiency) to 1:2,000,000 (prothrombin deficiency). These prevalences increase 8 to 10 times in countries where consanguineous marriages are customary. To establish the type and severity of symptoms in each coagulation defect, 237 patients with the inherited deficiencies of fibrinogen, factor II, factor V, combined factor V and factor VIII and factor X have been investigated. The most severe symptoms were found in patients with factor X and prothrombin deficiencies, with a relatively high frequency of joint and muscle bleeding. Severe bleeding manifestations like those in the gastrointestinal tract and central nervous system were relatively rare for all defects. Umbilical cord bleeding, typical of afibrinogenemia and factor XIII deficiencies, were relatively frequent also in prothrombin, factor V and factor X deficiencies. Mucosal type bleeding symptoms such as epistaxis was relatively frequent in fibrinogen and factor V deficiencies.

Gene mutations. We report the genetic alteration of 21 families with factor VII deficiency from predominantly Middle-East countries, where this deficiency has been poorly studied. Using screening techniques as SSCP and heteroduplex analysis of PCR products and sequence analysis of the abnormal fragments we identified 19 mutations of which 12 were novel and 7 have been previously reported. Of the previously reported mutations, those at Arg152, Arg304 and Thr359 involved a CpG dinucleotide that provides an explanation for identical mutations in diverse populations. However, for the Cys310Phe mutation present in both the Iranian and Italian population haplotype studies established the possibility of identity by descent. Of the 12 novel mutations, 9 were missense mutations, localized mostly in the catalytic domain but also in the Gla domain and EGF2 domain of factor VII. Also 3 novel and one previously reported splice sites mutations were identified in these patients. We found a novel homozygous (-2bp) deletion type mutation on pre-proleader sequence of factor VII gene in 5 year old Chinese boy with severe factor VII deficiency. This mutation leads to a complete lack of detectable plasma FVII reporting that this situation is not incompatible with human life. We report also the first case of an insertion type mutation in the factor VII gene that caused a severe plasma deficiency in a 5 year old girl from Oman with factor VII: C coagulant activity of less than 1% and factor VII antigen levels of 10%. This insertion consists of a duplication of codons 212 to 217, probably by slipped mispairing between 2 copies of a direct repeat (GCGAGCACGAC) separated by 4bp. In vitro study of the mutant recombinant protein by stable cell line using DHFR deficient CHO cells revealed a combined defect, i.e., intracellular degradation

in the preGolgi compartment associated with a secretion defect demonstrated by pulse-chase labelling experiments using ^{35}S methionine. Only small amounts of FVII with not detectable procoagulant activity were secreted into conditioned media.

These results verify both the hypothesis derived from molecular graphics analysis of FVIIa, and demonstrate that both a secretion and a functional defect is the mechanism whereby this insertion causes FVII deficiency. This mutation probably does not interfere with FVII synthesis, but is associated with various defects including abnormal folding, intracellular degradation, secretion failure and loss of coagulant activity. To our knowledge, this is the first instance of a FVII deficiency caused by a perturbation at its calcium-binding site in the catalytic domain.

On the whole, the novel factor VII gene mutations identified in this study extended by 30% the data base mutation analysis of the factor VII gene.

The spectrum of mutations in the ERIGC-53 gene associated with the combined deficiency of factor V and factor VIII was substantially enlarged by this study conducted in 35 families. All the 13 mutations identified are likely to results in the production of a truncated protein or no protein at all. Our study has also shown that some patients with the combined deficiency produce normal amount of ERGIC-53, indicating that other as yet unidentified molecular defects are the basis of combined factor V and factor VIII deficiency.

Management. The treatment of rare coagulation defects is usually simpler than that of the hemophilias, because of the bleeding tendency is less severe, lower hemostatic levels of deficient factor are needed and factor half-lives are usually long except for factor VII. The first requirement of replacement therapy is safety from transmission of bloodborne infectious agents and the second requirement is low cost, particularly in developing countries. Fresh frozen plasma is inexpensive has the advantage of containing all coagulation factors and can be virally inactivated with solvent/detergent.