
Factor IX is the zymogen of a serine protease which participates in the middle phase of intrinsic blood coagulation. Bovine factor IX is a single chain glycoprotein with a molecular weight of 55,000, upon activation by factor Xa(activated factor X) gives rise to factor IX(activated factor IX) having a two chain structure (mol wt 66,000) and an activation peptide (mol wt 9,000). (K. Fujikawa, M. E. Legaz, H. Kato and E. H. Dave, Biochemistry, 33, 4508 (1994)). A protease from Russell's viper venom (RVV) also activates factor IX by cleaving only one specific peptide bond (Arg-Val) (P.A. Lindquist, K. Fujikawa and E. H. Dave, Fed. Proc., 35, 1353 (1976)). Bovine factor IX(RVV) thus activated has a molecular weight of 55,000 identical to that of factor IX but is composed of two peptide chains held together by one or more disulfide bonds. After reduction and alkylation of disulfide bonds, two peptide chains were separated on a column of SP-Sephadex C-25 by a salt and pH gradient in the presence of 8 M urea. The sequence analysis of factor IX in progress on these two peptide chains using their subpeptides produced by cleavage at methionine, lysine, arginine and tryptophan. Preliminary results indicate that the sequence of factor IX is homologous to that of factor X and relabeled also to prothrombin and other vitamin K-dependent blood coagulation factors. A high degree of sequence homology is also observed between the heavy chain (the carboxyl-terminal half of the molecule) of factor IX and pancreatic serine protease such as trypsin, chymotrypsin and elastase.

GENETIC VARIANTS OF HEMOPHILLIA B. J.B. Roberts, K.B. Chung and J.C. Goldsmith University of North Carolina School of Medicine, Chapel Hill, North Carolina, U.S.A.

The purpose of this report is to describe genetic variants of hemophilia B. Most variants have been distinguished on the basis of clinical severity of their disease, as well as immunological, functional, and biochemical characterization of the Factor IX molecule. They have been classified according to the degree of cross-reactivity of the Factor IX molecule with specific homologous and heterologous anti-Factor IX antibodies using both inhibitor neutralization and radioimmunoassay techniques. Some hemophilia B variants have a Factor IX molecule that cross-reacts completely with anti-Factor IX antibodies. Other hemophilia B patients have a Factor IX molecule that has no detectable cross-reactivity with anti-Factor IX antibodies. A few Factor IX antibodies have been described in the literature. The Factor IX antibody has been studied extensively with a Factor IX molecule that shows complete cross-reactivity with anti-Factor IX antibodies. The Factor IX antibody has been shown to have 6 to 10% activity and 20% antigenic activity. This variant Factor IX molecule shows delayed activation in the presence of partially purified Factor IX and CA2. Although it is otherwise similar to normal human Factor IX, it is significantly different from normal human Factor IX in several respects. These patients have an abnormal Factor IX molecule that is recognized antigenically, but not functionally.

HEMOPHILLIA B: GENETIC VARIANTS AND CARRIER DETECTION. C.E. Kasper, B. Osterud, S. I. Harengsr. School of Medicine, Univ. of Southern California; Orthopaedic Hospital, Los Angeles; School of Medicine, Univ. of California at San Diego; J.A. Hospital, San Diego; U.S.A.

In 92 males with hemophilia B from 71 kindreds, we measured factor IX activity, procorbin time using bovine thromboplastin (bovine tpa), and Factor IX antigen both by inhibitor neutralization using a human Factor II inhibitor and by electroimmunoassay using a precipitating rabbit anti-human-factor IX antiserum. Eighty patients with 25 or less Factor IX activity could be divided into 4 groups: (1) 7 patients with greatly prolonged bovine tpa times and normal levels of Factor IX antigen; (2) 17 patients with mildly prolonged bovine tpa times and factor IX antigen levels between about 25% and normal; (3) 8 patients with normal bovine tpa times and antigen levels between about 25% and normal; (4) 48 patients with normal bovine tpa times and no measurable antigen excess. Some of the latter group were also treated with a rabbit anti-human-factor IX antisera and no antigen was found. None of 12 patients with mild hemophilia B (factor IX activity of 4 to 22%) had a prolonged bovine tpa time although 4 patients had decreased Factor IX antigen over activity. Thus, about 1/3 of these 92 hemophilia B patients had evidence of an abnormal factor IX molecule. Factor IX activity was also measured in 48 normal women and in 51 definite carriers of severe hemophilia B. Probability curves were derived to estimate the chance of a woman being a carrier based upon her factor IX level and her degree of kinship to a definite carrier. The relation between factor IX activity and antigen was also delineated for normal women and for carriers. In kindreds in which affected males had excess antigen, some carriers could be distinguished from normal women on the basis of excess antigen over activity. In appropriate kindreds, prolonged bovine tpa times help distinguish some carriers.