Hereditary Factor VII Deficiency: Heterogeneity Defined by Combined Functional and Immunochemical Analysis

By Douglas A. Triplett, John T. Brandt, Mary Ann McGann Batard, Janis L. Schaeffer Dixon, and Daryl S. Fair

Twenty-six patients with hereditary factor VII deficiency (VII:C < 10%) were evaluated using a panel of three thromboplastins of varying species and tissue origin in both coagulant and chromogenic assay systems. Normal values for the coagulation and chromogenic assays were 104% ± 7% and 108% ± 21%, respectively. Factor VII antigen was measured by a specific radioimmunoassay (normal, 470 ± 112 ng/mL). The patients were divided into two groups based on the factor VII:Ag assay results. Group 1, 18 patients, had decreased levels of factor VII:Ag and group 2, eight patients, had normal levels of factor VII:Ag. The two groups were further subdivided on the basis of discrepant factor VII:C levels in the chromogenic and coagulant assays. The number of observed patterns of functional factor VII:C activity suggests a high degree of complexity of factor VII and thromboplastin interaction. Whereas no clinical bleeding was reported in any of the nine black patients evaluated, all Caucasians (16) and one Hispanic presented with mild to severe bleeding. Patients with factor VII:C > 10% using a human thromboplastin had a negative bleeding history, regardless of the activity measured with other thromboplastins. Factor VII activity measured with a human thromboplastin appears to correlate best with the clinical picture.

MATERIALS AND METHODS

Patients. Each of the 26 patients had been previously diagnosed and thoroughly evaluated at various medical centers (Table I). All were diagnosed as hereditary factor VII deficiency with <10% factor VII:C activity determined by an assay using rabbit tissue thromboplastin. The plasma samples were kindly provided by the following investigators: James Baker, Long Beach (Calif) Memorial Hospital; Walter Bowie, Mayo Clinic, Rochester, Minn; Fred Dombrose, University of North Carolina, Chapel Hill; Rodger Edson, University of Minnesota, Minneapolis; Bruce Evatt, Centers for Disease Control, Atlanta; Lyman Fisher, Medical College of Virginia, Richmond; Helen Glueck, University of Cincinnati; George King, George King Biomedical, Overland Park, Kan; Jessica Lewis, University of Pittsburgh; Roland Lonser, Hinsdale (Ill) Hospital; Fred Lucas, Cleveland Clinic; Mary Ann Miller, University of Cincinnati; John Penner, Michigan State University, East Lansing; Samuel Rapaport, University of California, San Diego; Oscar Ratnoff, Case Western Reserve University, Cleveland; Louis Verdi, Philadelphia; Robert Weetman, Indiana University, Indianapolis; Harvey Weiss, Columbia University, New York; and Richard Wertz, Sutter Memorial Hospital, Sacramento, Calif. With few exceptions, normal plasma samples were shipped with the patient samples.

Blood samples were obtained using standard collection techniques: each sample was drawn into 0.129 mol/L sodium citrate and the plasma separated after centrifugation at 2,000 g for ten minutes. The platelet-poor plasma was snap frozen at -70 °C. Transport of samples from the referring laboratories was carried out on dry ice. Attempts were made to minimize cold activation by storing plasma at -70 °C in plastic containers. Pooled normal plasma (PFP) for controls was prepared using equal amounts of plasma from 20 healthy donors (ten males and ten females).

Factor VII:C coagulant assay. Coagulant factor VII:C assays were performed according to previously reported techniques, carefully following the instructions of each thromboplastin manufacturer.12 Factor VII-deficient substrate plasma was purchased from General Diagnostics (Morris Plains, NJ). The thromboplastins used in both the coagulant and the chromogenic factor VII:C assays included rabbit brain (Dade, Miami), simian-rabbit brain (Stago-
Biochemica Laboratories, Asnieres, France), and human brain
(British Comparative Thromboplastin, gift from Dr L. Poller, With-
ington Hospital, Manchester, England). The coagulation assays
were performed on a Fibrometer (BBL, Cockeysville, Md). Values
were considered normal if they were within two standard deviations
of the normal distribution mean, and abnormal if below two standard
deviations.

Factor VII:C chromogenic assay (amidolytic assay). The chro-
monic VII:C assay was a modification of a previously reported
assay and was performed as follows\textsuperscript{13}: to 100 \mu L of plasma diluted
with TBS (Tris-buffered saline, containing 0.05 mol/L Tris, 0.125
mol/L \textit{NaCl}, pH 7.5, 37 °C) was added 25 \mu L of 0.025 mol/L
\textit{CaCl}\textsubscript{2}, and 50 \mu L thromboplastin. The mixture was incubated at
37 °C for one minute and then 50 \mu L of purified human factor II
(3.10 U/ml, gift of Dr William Lawson, State of New York
Department of Health) was added. The mixture was incubated at
37 °C for exactly three minutes. The activation of factor II was
terminated by adding 50 \mu L of 0.15 mol/L EDTA, and the sample
was cooled on ice. To determine the quantity of factor Xa formed, a
100-\mu L aliquot of this mixture was added to 600 \mu L of TBS and 100
\mu L of 1.0 mg/mL S-2222 (N-benzoyl-L-isoleucyl-L-glutamyl-
glycyl-L-arginine-p-nitroanilide, Kabi Vitrum, Malmö, Sweden),
and the rate of change in absorbance at 405 nm was measured. To
compensate for the color of the plasmas and the turbidity of the
various thromboplastins, a blank was prepared for each test, using all
the reagents in the appropriate dilutions with the exception of
S-2222. A second blank without exogenous factor X was also
determined. Factor VII:C was calculated from a dilution curve
prepared each day from PNP-diluted 1:100, 1:200, 1:400, 1:800,
and 1:1,000 in TBS. The reported results represent the mean deter-
mination of at least duplicate testing of two dilutions of the sample
patient.

Factor VII:Ag radioimmunoassay. Factor VII antigen was
determined by an equilibrium double antibody radioimmunoassay as
described previously.\textsuperscript{11} All of the specificity, nativity, and affinity
controls required of a radioimmunoassay were fulfilled by the assay.
The factor VII:Ag concentrations were measured using purified
human factor VII as the standard. The range of sensitivity of the
assay was between 1 and 500 \mu g factor VII:Ag per milliliter and the
coefficient of variation was from 1% to 3% within assay and from
12% to 16% between assays. Purified human factor VII and plasmic
factor VII from normal and patient plasmas inhibited competitive
assays with parallel slopes, indicating their immunochemical iden-
tity and validating the measurement of factor VII:Ag in plasma.
Quantification of factor VII:Ag is given in nanogram per milliliter
and percentage of the normal mean concentration.

Briefly, the radioimmunoassay was conducted in the following
manner: 250 \mu L of \textsuperscript{125}I-factor VII (5 \times 10^{-10} \text{ mol/L}), 250 \mu L of a
dilution of purified human factor VII (or plasma as the competing
antigen), and 250 \mu L of rabbit antihuman factor VII (diluted
of significant functional factor VII activity allowed further analysis. The precipitates were formed after addition of goat antirabbit IgG and incubation for six hours at 4 °C. The precipitates were collected by centrifugation at 2,000 g for 15 minutes at 4 °C, and the amount of factor VII activity in the supernatant was counted using radioimmunoassay. The buffer used in the assay and for all dilutions was 0.025 mol/L borate buffer, pH 8.3, containing 0.025 mol/L NaCl, 2% of heat-inactivated normal rabbit serum, 10 mmol/L benzamidine, 100 U/mL TrasyloL, and 1 mmol/L phenylmethylsulfonyl fluoride. All sera were heat inactivated before use, and the goat antirabbit IgG serum contained 10 mmol/L benzamidine.

RESULTS

The concentration of factor VII:Ag in normal plasma and patient plasma samples was determined using the radioimmunoassay. The mean concentration of factor VII:Ag in normal plasma was previously determined to be 470 ± 112 ng/mL. The range of values in the patient samples was from 7 to 532 ng/mL, with a mean of 182 ng/mL. Eighteen patients with decreased factor VII:Ag levels were classified as group 1 (Table 1). The remaining eight patients had normal factor VII:Ag that fell within two standard deviations of the mean. These patients were classified as group 2, i.e., having a normal factor VII antigen concentration (Table 1). There were no cases in which factor VII antigen was not detected.

Very low levels (<10%) of factor VII:C were measured by all functional assays in 20 of 26 patients (Table 1). Higher levels of functional activity (>10%) were observed in the remaining six plasma in at least two assays. Two patients (Nos. 10 and 13) showed >10% factor VII:C activity with a single assay system, using human brain thromboplastin. However, factor VII:C activity of >10% in a single assay was not considered significant because it may have reflected intrinsic variation within the assay procedure. The presence of significant functional factor VII activity allowed further subclassification of the patients; group 1 was divided into 17 patients with reduced antigen levels and <10% factor VII activity (1A) and one patient with reduced levels of factor VII antigen and significant levels of functional factor VII activity (1B). Group 2 was similarly divided into three patients with normal levels of antigen and no significant functional factor VII activity (2A) and five patients with normal levels of factor VII antigen and significant levels of functional factor VII activity (2B).

For a given thromboplastin, the results were usually similar between the two types of functional assay systems (chromogenic and coagulant). This correlation suggests that spurious elevated coagulant activity secondary to in vitro cold activation was not a factor in the study. However, the results of patient No. 18 raised the possibility of some cold activation because the coagulant activity using human thromboplastin was significantly greater than the corresponding chromogenic assay determination.

Previously, Girolami et al.14 suggested that differences in factor VII coagulant activity observed with different thromboplastins should be taken into account when describing variant forms of factor VII deficiency. Differences of >15% in factor VII:C activity among various thromboplastins were categorized as discordant results in our patient population, as suggested by Girolami et al.14 Such results were seen in five patients (Nos. 18 and 23 through 26), including the one patient from group 1B and four of five patients from group 2B. A striking example of such a functional variant is patient No. 18, whose factor VII:C was <3% with a rabbit thromboplastin but 90% with the human thromboplastin (Table 1). In each of these cases, the highest apparent factor VII:C activity was seen with human thromboplastin. Of interest, none of these patients had a bleeding tendency and one had a thrombotic tendency (No. 26).

This study included 16 Caucasian patients, nine black patients (Nos. 3, 10, 18, 20, 22, and 23 through 26), and one Hispanic patient (No. 21). A bleeding history was obtained in 0/9 black patients, 16/16 Caucasian patients, and 1/1 Hispanic patient. Six of the eight patients in group 2 were black, including all five patients in group 2B. In contrast, 15 of 16 Caucasian patients were in group 1, and all had bleeding manifestations.

DISCUSSION

Our study represents a comprehensive laboratory evaluation of hereditary deficiency of factor VII. The results confirm and expand the heterogeneity of factor VII deficiency suggested by previous investigations.8-10,15-17 Of particular interest was the relatively high number of patients with normal factor VII antigen levels and absence of any patient with undetectable factor VII antigen. These data suggest that factor VII deficiency frequently is characterized by a variant, functionally altered molecule. The patients could be divided into two major groups based on factor VII antigen levels, and each of these major groups could be subdivided based on presence of significant levels of functional factor VII activity.

The patients in groups 1A and 2A would have been classified as classic factor VII deficiency by traditional coagulation assays. The results of the antigen analysis would suggest that there is a previously unrecognized heterogeneity even in this group. The patients in group 1B and 2B are of particular interest because they show varying reactivity, depending on the species of origin of the thromboplastin. Each of these variant molecules was characterized by increased functional activity in the presence of a human-derived thromboplastin; corresponding to this was the absence of any bleeding history in these patients. These findings suggest that evaluation of patients with factor VII deficiency using a human thromboplastin would be helpful in predicting risk of bleeding tendency.

However, the major determinant of clinical bleeding in this study appeared to be racial origin. None of the nine black patients evaluated had a history of bleeding, regardless of the type of defect (two group 1A, one group 1B, one group 2A, five group 2B). On the other hand, all 16 Caucasian patients and the one Hispanic patient reported bleeding problems ranging from mucocutaneous bleeding to severe recurrent hemarthroses and intracerebral hemorrhage. Of further interest was the fact that all five of the patients in
group 2B were black, suggesting much higher incidence of functional variants in this segment of the population. The one patient who displayed a thrombotic tendency, a previously recognized association with factor VII deficiency, was black and showed increased reactivity with human thromboplastin (No. 26).34

This study represents an initial approach to the characterization of the molecular defects in hereditary factor VII deficiency. The results indicate that there is a high incidence of variant, nonfunctional molecules in this disorder and, as suggested by previous studies, a relatively high frequency of functional variant molecular defects.6–10 Using this approach, it may be possible to identify additional patients with functional variants of factor VII. These individuals would be of particular interest for unraveling the molecular biology of factor VII. In addition, this approach may be particularly valuable in family studies and in identifying the doubly heterozygous patient. Further studies to isolate and characterize the defective molecule in patients with functional variants are being pursued.

REFERENCES

Hereditary factor VII deficiency: heterogeneity defined by combined functional and immunochemical analysis

DA Triplett, JT Brandt, MA Batard, JL Dixon and DS Fair