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STUDY OF IIA VON WILLEBRAND'S DISEASE (VWD) VARIANTS TO DETERMINE DEGREE AND TYPES OF HETEROGENEITY. S.M. Enayat, F.G.H. Hill, *Y. Sultan and C.W. Williams. Haematology Department, The Children's Hospital, Birmingham, U.K. *Hemostase et de Thrombose, L'Hopital Cochin, Paris, France.

Thirty four IIA vWD patients (16 from kindred I, 2 from kindred II and 17 unrelated patients) from 19 families were studied to compare multimer patterns using discontinuous SDS gel electrophoresis on a variety of agarose gels. Platelet multimers and effect of EDTA on plasma multimers were also studied in some.

The large kindred and 9 other patients showed identical multimer and triplet abnormalities. The 11 other patients showed different multimer patterns either by having intermediate multimers or different triplet patterns. The second kindred had a similar triplet abnormality to kindred I but had intermediate multimers. Two other patients showed similar patterns except on 2% agarose gels when differences in the lowest multimer was seen. Of the 3 patients of YS, one showed the common IIA pattern but also had intermediate multimers, another had an unusually faint upper triplet band, while the third in addition to a faint upper triplet band with ESVWF 2^{+} had no identification of minor or major bands with ESVWF 10^{+} Another patient lacked high and some intermediate multimers but had a normal triplet pattern. The pattern we have seen in Kernoff's patient (1) still appears unique. In kindred II abnormal triplets persisted and high multimers appeared in EDTA plasma. In kindred I (and similar patients) intermediate multimers and a change in triplet pattern was observed in EDTA while lysed platelets showed an abnormal pattern different to the plasma one.

This emphasizes the heterogeneity of IIA vWD and the need to consider multimer deletion, triplet pattern, platelet multimers, effect of EDTA in trying to subclassify in order to study structure function relationships of vWF.

1. Kernoff PBA, Gruson R, Rizza CR. (1974) A variant of factor VIII related antigen. Br. J. Haematol. 26: 435.

$^{+}$ESVWF 2 and 10 are monoclonal antibodies to vWF epitopes.

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CALPAIN AND ELASTASE ARE NOT RESPONSIBLE FOR THE VON WILLEBRAND FACTOR FRAGMENTS IN NORMAL PLASMA AND IIA VON WILLEBRAND DISEASE. S.D. Berkowitz (1), H. Nozaki (2), K. Titani (2), T. Murachi(3), and T.S. Zimmerman (1). Scripps Clinic and Research Foundation, La Jolla, CA, U.S.A (1), University of Washington, Seattle, WA, U.S.A (2), and Kyoto University, Kyoto, Japan (3).

Recent evidence suggests that proteolysis plays an important role in some forms of inherited and acquired von Willebrand disease (vWD). Using monoclonal epitope mapping, we have examined the proteolysis of the von Willebrand factor (vWF) subunit with platelet calcium activated neutral protease (CANP) and human leukocyte elastase and found that they are not responsible for the proteolytic cleavage seen in normal individuals and IIA vWD. Previously we have shown that in vivo proteolysis of vWF is a normal event with a small but consistent proportion of plasma vWF being composed of 189, 176, and 140 kD fragments cleaved from the 225 kD subunit. In IIA vWD the proportion of cleaved vWF is increased. Because calcium activated neutral protease (CANP, calpain) and one or more enzymes released from polymorphonuclear leukocytes are known to proteolyze vWF in vitro with resultant loss of large multimers similar to that seen in IIA vWD, they have been suggested as being responsible for the proteolysis in vivo. We have now digested highly purified vWF with porcine CANP I and II and performed monoclonal epitope mapping on the resulting fragments. We found no difference in the size, location, and quantity of the fragments produced by calpain I versus calpain II. New fragments were detected of approximately 200, 170, 150, and 125 kD. There was no evidence for generation of the native fragments. Mapping of the 170 and 150 kD calpain-cleaved fragments revealed them to be from different parts of the molecule than the native 176 and 140 kD fragments. Digestion of vWF with human leukocyte elastase produced new fragments at 210/205, 190, 165, 145/140, and 130/125 kD. No generation of native fragments was detected. Monoclonal epitope mapping of the 190 and 145/140 kD elastase-cleaved bands proved that they come from opposite ends of the vWF molecule than the native 189 and 140 kD fragments, respectively. Therefore, CANP and human leukocyte elastase do not produce the proteolyzed fragments present in normal and IIA vWD and probably do not cause the loss of large multimers seen in that disorder.

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TYPE IIB VON WILLEBRAND DISEASE WITH CHRONIC THROMBOCYTOPENIA : BENEFICIAL EFFECT OF CRYOPRECIPITATE SUPERNATANT INFUSION ON PLATELET COUNT AND BLEEDING. **A. Derlon, A. Le Querrec, E. Lebrun, G. Tobelem*, M. Thomas. Laboratoire d'Hématologie, C.H.U. Côte de Nacre 14040 CAEN. *Unité INSERM 150, Hôpital Lariboisière, 75010 PARIS FRANCE.**

As we previously described, plasma infusion increased platelet count (PC) in four patients with IIB von Willebrand disease with severe thrombocytopenia. In a sixty years old patient in the same family, with chronic thrombocytopenia (PC = 30 000/ml) associated to an absence of large von Willebrand Factor multimers (vWF) in plasma, we successfully treated :

1° A gastrointestinal bleeding episode with fresh frozen plasma infusion (15ml/Kg/day).

2° Three months later a severe epistaxis with cryoprecipitate supernatant (15ml/Kg/day).

During these bleeding episodes, the efficiency of these two treatments on the PC could be ascertained according to the following figure

PLATELET COUNT/ml during treatment

	Plasma infusion	Cryoprecipitate supernatant infusion
D0	30 000	30 000
D5	70 000	120 000
D10	100 000	150 000
D13	170 000	180 000

We observed after ten days of these two treatments the following biological effects : a normalisation of vWF cross immunoelectrophoresis, of ristocetin induced normal platelet aggregation by patient's plasma, and of patient's plasma vWF binding to control platelets.

In conclusion a factor appears to be present in both fresh frozen plasma and cryoprecipitate supernatant which prevents the abnormal binding of von Willebrand Factor (in this IIB von Willebrand disease) to the patient's platelets.

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A VARIANT OF VON WILLEBRAND'S DISEASE (vWD) WITH IIB-TYPE MULTIMER PATTERN IN THE ABSENCE OF ENHANCED PLATELET AGGLUTINATION. S.E.Cottrell, R.T.Wensley, A.M.Burn and I.W. Delamore. Department of Clinical Haematology, Royal Infirmary, Oxford Road, Manchester, M13 9WL, U.K.

A 62 year old man with vWD has suffered from repeated episodes of melaena - his son and daughter have inherited the disorder with few symptoms so far. Laboratory findings in them include consistently prolonged bleeding times, normal factor VIII coagulant activity and decreased ristocetin cofactor activity. Levels of von Willebrand factor (vWf) antigen were lower measured by immunoradiometric assay than by Laurell immunoelectrophoresis. Analysis of vWf structure by SDS agarose gel electrophoresis showed loss of only the large multimers in plasma and the triplet structure of the smaller multimers showed sub-bands more intense than normal. However, the platelets contain the whole series of multimers with a similar pattern to normal. This was suggestive of type IIB vWD but agglutination of the patients' platelet-rich plasma with low concentrations of ristocetin was not enhanced. Agglutination was reduced compared to normal platelet-rich plasma at a final ristocetin concentration of 1 and 2 mg/ml and absent at a final concentration of 0.5 mg/ml. Platelet-type vWD was eliminated as addition of normal plasma or cryoprecipitate to patient's platelet-rich plasma did not produce spontaneous aggregation. The patient has never had thrombocytopenia. We feel that this family demonstrates further the heterogeneity of vWD.