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ANALYSIS OF THE VON WILLEBRAND FACTOR (vWF) GENE IN 6 PATIENTS WITH SEVERE TYPE III VON WILLEBRANDS DISEASE
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DNA from 6 unrelated patients with severe type III von Willebrands disease (vWF antigen < 0.01u/dl) was studied with a cDNA probe for the 3' end of the vWF gene. DNA was extracted from peripheral blood leucocytes using standard techniques and was digested with a range of restriction enzymes. DNA fragments were separated by electrophoresis in 0.7% agarose and were southern blotted onto hybond-N (Amersham). The probe used was pvWF1100, a 1.1kb PstI fragment derived from the 2.28kb vWFcDNA insert of pvWF2280 isolated from a human endothelial cell cDNA expression library (Verweij et al, Nucleic Acids Res 13 (1985) 4699-4717). The probe corresponds to nucleotides 7083 to 8191 of the vWF cDNA (first nucleotide of initiator methionine as 1).
When digested with BglII and probed with pvWF11000, normal DNA showed two invariant bands (13 and 4.9kb) and polymorphic bands of 9 and/or 7.4kb. This pattern was also seen in 5 of the 6 severe vWD patients DNA suggesting that in this 3' area of the gene they had no major deletions or rearrangements. In the 6th case however the band of 4.9kb was not seen and did not appear to be replaced by any novel fragments, suggesting a partial deletion including some of the 3' end of the gene. This patient had the clinically severest form of the condition in that the patient had developed, some 10 years ago, an antibody (inhibitor) to vWF as detected by the ability of the patients plasma to inhibit restocetin cofactor activity in normal plasma. His parents were related (his mother was his father's second cousin) and had levels of vWFAg, considerably lower than those of factor VIII activity. This situation has been previously reported in carriers of recessive severe vWD. vWD was also present in a second family member, but in a less severe form (vWFAg 3u/dl). This patient and all other members of the family have, to date, given normal restriction fragment patterns with the vWF probe and several enzymes, including BglII.

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VON WILLEBRAND'S DISEASE TYPE IIB ASSOCIATED TO A COMPLEX THROMBOCYTOPENIC THROMBOCYTOPATHY: J. Batlle (1), M.F. López-Fernández (1), C. López-Berges (1), R. Sánchez (2), L.G. Villarón (2), and T.S. Zimmerman (3). Dept. Hematology (1) and Internal Medicine (2), Hospital Clinico, Universidad de Salamanca, Spain and Dept. Basic & Clinical Research, Scripps Clinic & Research Foundation, La Jolla, CA. (3).

A family bleeding disorder characterized by a new association between Type IIB von Willebrand's disease (vWD) and a complex platelet disfunction, with an intermittent thrombocytopenia is described in two patients from the same generation. The mother and a maternal aunt died having severe bleeding diathesis. The platelet abnormalities included: borderline or slightly low platelet count but moderate thrombocytopenia coincedent with the acute bleeding episodes, giant platelet site with a very heterogeneous distribution width, large number of vesicles in platelets by electron-microscopy recalling the "Swiss-Cheese" platelets, abnormal platelet aggregation induced by ADP, collagen, epinephrin and slightly, by thrombin, defective release of ^{14}C-Serotonin, von Willebrand factor (vWF) and platelet factor 4 induced by thrombin or ADP. DDAVP was given to both patient and a partial and transitory correction of bleeding time, thrombocytopenia, presence of platelet aggregates on smear besides a brief appearence of larger multimers of vWF and an increase in all Factor VIII/von Willebrand Factor (FVIII/vWF) properties were seen. Binding of labeled vWF using radiolabelled monoclonal anti-vWF antibody showed an enhanced binding of the patients' vWF, induced by ristocetin, to either normal or patients' platelets. In contrast, the binding of labeled purified normal vWF induced by thrombin to patients' platelets was decreased as compared with the correspondent control. Thus, both patients have platelet disfunctions characteristic for more than one specific platelet disorder. Several associations between platelet and FVIII/vWF abnormalities have been described. This is the first family presenting association of Type IIB vWD and a complex thrombocytopathy. The inherited or acquired (induced by the abnormal IIB vWF platelet interaction) nature of the abnormality is discussed.

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THE PLATELET AGGREGATING PROPERTIES OF TYPE IIB VON WILLEBRAND FACTOR (vWF): THE ROLE OF PLATELET ACTIVATION, FIBRINOGEN AND TWO DISTINCT MEMBRANE RECEPTORS. L. De Marco (1), M. Mazzucato (1), M.G. Del Ben (1), U. Budde (2), A.B. Federici (3), A. Girolami (4) and Z.M. Ruggeri (5). Centro Immuno-trasfusionale, U.S.L. n. 11 Pordenone, Italy (1), Universitats-Krankenhaus Eppendorf, Hamburg, W. Germany (2), Centro Emofilia, Milano, Italy (3), Patologia Medica II, Padova, Italy (4) and Scripps Clinic, La Jolla (Ca), U.S.A. (5).

Three preparations of purified von Willebrand factor (vWF), obtained from unrelated patients affected by type IIB von Willebrand disease, were found to have normal sialic acid content (between 129-170 nmoles/mg of vWF, as compared to 158 ± 17 nmoles/mg in four normal preparations) and to induce platelet aggregation in the presence of physiologic levels of divalent cations and without addition of ristocetin. A monoclonal antibody that blocks the vWF binding domain of the platelet glycoprotein (GP) Ib caused complete inhibition of IIB vWF-induced aggregation. On the contrary, a monoclonal antibody that blocks the receptor for adhesive proteins on the platelet GPIIb/IIIa complex failed to inhibit the initial response of platelets to high concentration of IIB vWF Moreover, IIB vWF caused agglutination of formalin-fixed platelets that was blocked only by the anti-GPIb antibody, suggesting that the binding of vWF to GPIb, even in the absence of ristocetin, results in platelet-platelet interaction that is followed by exposure of the GPIIb/IIIa receptors for adhesive proteins. Endogenous ADP, normally active platelet metabolism and fibrinogen binding to GPIIb/IIIa were necessary for maximal and irreversible platelet aggregation. In the absence of fibrinogen, however, aggregation was mediated by vWF binding to GPIIb/IIIa. A 52/48 kDa tryptic fragment containing the GPIb binding domain of normal vWF completely blocked the aggregation induced by all three IIB vWF preparations. The present study defines in detail the mechanisms involved in IIB vWF-induced platelet aggregation. Moreover, it establishes that the GPIb binding domain of normal and IIB vWF are closely related and that desialylation is not required for the direct interaction of IIB vWF with GPIb.

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EVIDENCE FOR AN ABNORMAL EXPRESSION OF THE COLLAGEN BINDING DOMAIN IN VON WILLEBRAND'S DISEASE TYPE II.
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A recently developed new series of monoclonal antibodies (MAbs) against the von Willebrand factor (vWf) included antibodies strongly inhibiting (Mab vWf-41) and partly inhibiting (Mab vWf-33) the collagen binding of vWf. We also characterized two Mabs with interacting properties against the ristocetin induced platelet aggregation (MAbs vWf-21 and vWf-39). These antibodies were conjugated with horse-radish peroxidase (HRP) and examined in different constructions forming two-site MAb ELISA's for plasma vWf:Ag and compared with polyclonal antibody ELISA. Symmetrical MAb-ELISA (i.e. same Mab for extraction and detection) gave practical no dose-response in the standard assay, whereas any different combination of Mabs gave favourable dose-response relationships in sensitive ELISA's for vWf:Ag. Two different sandwiches were chosen using MAb vWf-33 and Mab vWf-41 at either side of the ELISA. These two assay models gave results of plasma from normal persons almost identical to those obtained with polyclonal antibody ELISA. Also in type I von Willebrand's disease these three assays performed very uniformly. In subtypes II plasma (IIA: n=7; IIB: n=3, IIC: n=1, IID: n=1) . the assay using vWf-33 for coating and vWf-41-HRP for detection measured considerably lower than the polyclonal ELISA and the Mab-ELISA based on the opposite combination. We believe, that our results are indicative of a molecular defect in the collagen binding domain of vWf in subtype II plasma.