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Abnormal Proteolytic Degradation of Von Willebrand Factor After Desmopressin Infusion in a New Subtype of Von Willebrand Disease (IID)

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We describe two members of a single family, father and son, with mild factor XII deficiency associated to von Willebrand disease (vWD) with aberrant structure in whom distinct multimeric abnormalities and an abnormal proteolytic processing of von Willebrand factor (vWF) after desmopressin (DDAVP) administration were present. They had a mild bleeding history, low levels of vWF-related activities, and a prolonged bleeding time. Low-resolution agarose gel electrophoresis showed a vWF with all size multimers in plasma and platelets. Higher-resolution agarose gels demonstrated that the main band was present, but the relative proportion of the satellite bands was markedly reduced. The smallest oligomer was not increased. After the infusion of DDAVP to the father, a transient increase in the relative proportion of the satellite bands was seen, as described in normal individuals. No difference in the structure of vWF was observed when blood was collected with proteinase inhibitors. The analysis of native subunits of vWF and their proteolytic derived fragments, after DDAVP administration, showed a temporary augmentation of the 176 kDa fragment, as seen in normal subjects, as well as an increase of the 189 kDa fragment. This finding had not been reported previously either in normal individuals or in patients with vWD.

Key words: desmopressin, proteolysis, WF

INTRODUCTION

We have previously reported that the infusion of desmopressin (DDAVP) in normal individuals is followed by a proteolytic degradation of von Willebrand factor (vWF) [1]. After DDAVP administration, multimeric analysis of vWF shows a transient increase in the relative proportion of satellite bands, and subunit analysis of vWF shows a temporary decrease in the relative proportion of the intact native 225 kDa subunit and of the 189 and 140 kDa-derived fragments, as well as an increase in the relative proportion of the 176 kDA-derived fragment. The subunit composition in von Willebrand diseases types IIA and IIB indicates a presence of increased proteolysis of vWF. In contrast, in variants with aberrant multimeric structure, a reduced cleavage of the vWF subunit is seen [2]. We describe here two members of the same family with a history of mild lifelong bleeding transmitted, probably, as an autosomal dominant trait. Both had a mild factor XII deficiency associated to vWD. Their vWF showed an aberrant structure as well as an abnormal pattern of proteolysis of vWF after DDAVP infusion. We also compare this new variant with a patient with a heterozygous type IIC vWD previously reported [3], who although sharing some multimeric abnormalities, showed a different proteolytic degradation patttern after DDAVP administration.

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MATERIALS AND METHODS

The patients and normal controls included in this study were all medication-free for at least 10 days before the measurement of skin bleeding time and/or before drawing venous blood; 1-deamino-8-D-arginine-vasopressin (DDAVP, Minirin, Ferring Laboratories, Malmo, Sweden) was infused to the father, a normal individual and a patient with heterozygous type IIC vWD, at a dose of 0.4 μg/kg with blood samples obtained before and at various times after the infusion.

Proteinase inhibitors included leupeptin, EDTA, N-ethylmaleimide (NEM), aprotinin, and phenylmethylsulfonylfluoride (PMSF) (Sigma Chemical Co., St. Louis, MO). Electrophoresis-pure reagents were acquired from Bio-Rad, Richmond, CA. Agarose HGT(P) from Seakem (Marine Colloids, FL) and Sigma VII (Sigma Chemical Co., St. Louis, MO) were used. Rabbit antimouse IgG was from Zymed (South San Francisco, CA). All other reagents were of the highest grade available.

METHODS

The methods for blood collection and preparation of platelet-rich plasma (PRP) and platelet-poor plasma (PPP), as well as for the determination of ristocetin-induced platelet agglutination (RIPA) in PRP, were as previously described [3]. Plasma with proteinase inhibitors was obtained in the presence of 1 mM leupeptin, 6 mM NEM, and 5 mM EDTA [2]. Platelets were washed free of plasma constituents and then lysed according to previously published procedures [4,5]. The activity of von Willebrand factor antigen (vWF:Ag) by Laurell technique, ristocetin cofactor (vWF:RCo), and RIPA was assayed as explained elsewhere [6]. Bleeding time (BT) was measured by using Simplate II, General Diagnostics, Anaheim, CA. Factor VIII procoagulant activity (VIII:C) and factor XII were measured in plasma samples by a one-stage method based on the partial thromboplastin time [7]. The results of the assays were expressed in units per milliliter by using a standard plasma pooled from 30 healthy donors. For VIII:C the normal pool was calibrated in international units against the International Standard for FVIII and vWF, obtained by courtesy of Dr. Barrowcliffe (National Institute of Biological Standard and Control. Hampstead U.K.).

SDS-Agarose electrophoresis (short and long gel techniques) for analysis of vWF multimers was performed as according to our previous publication [6].

Subunit Analysis of vWF

For subunit analysis, immunoisolation of plasma vWF was carried out as already published [2,6]. vWF was isolated by incubation of 1–5 ml of plasma with 1–2.5 ml of agarose beads covalently linked with anti-vWF monoclonal antibody. After extensive washing, the vWF was eluted with 2 ml of 2% SDS, 0.1 M TRIS (pH 8.0) at 6°C and the eluate was concentrated using Amicon Centricon concentrations to a final volume of approximately 100 μl. SDS-polyacrylamide gel electrophoresis (PAGE) was done according to Laemmli [8]. An outline of the immunoblotting procedure is as follows: After SDS-5% PAGE, reduced immunoisolated vWF (5 μg/lane) was transferred to a sheet of nitrocellulose and detected by using a pool of 55 anti-vWF monoclonal antibodies, all of which reacted with the reduced 225 kDA subunit, as already described [2]. Sheets of nitrocellulose were reacted with ^{125}I-labeled rabbit antimouse IgG and, finally, submitted to autoradiography at −70°C. Each band was identified, excised, and counted in a gamma scintillation counter (Clinigamma, LKB, Bromma, Sweden), and its relative proportion was calculated. Every plasma sample from each patient was tested three times in this way and the arithmetic mean and range of the observed values were calculated.

RESULTS

Only the father (propositus) and the son of the family described here was studied; unfortunately no other members were available for this study. The propositus was a 27-year-old man. He experienced occasional episodes of epistaxis, prolonged hemorrhage following dental extractions. He required a blood transfusion after tonsillectomy when he was 14. His son was a 3-year-old boy who experienced ecchymosis after minor trauma as well as bleeding from the gums, otorrhagia, and occasional epistaxis. Usually the abnormal bleeding responded well to antifibrinolytic agents as well as DDAVP administration (intranasal). A maternal aunt and maternal cousin of the propositus also had hemorrhages after dental extractions and recurrent epistaxis, respectively. Consanguinity was not discovered and no other relevant anamnestic data could be elicited in this family.

The results of pertinent parameters of hemostatic function are reported in Table I. APTT was prolonged in both patients but it was corrected when patients' plasma was mixed with normal plasma. Both patients showed a prolonged bleeding time on the two occasions they were tested. They had decreased RIPA and low levels of VIII:C, vWF:Ag, and vWF:RCo. A certain degree of disproportion between the plasma levels of vWF:RCo and vWF:Ag was seen in resting conditions, the former being lower. Factor XII was always low in the son and on one occasion in the father. After DDAVP administration to the father the bleeding time became normal and a good response of VIII:C, vWF:Ag, and vWF:RCo was observed with disappearance of the disproportion seen at the basal state.

TABLE I. Clinical Data of the Patients

Patient	Platelet count ($\times 10^9$L)	Bleeding time (min)	APTT (sec)	RIPA	VIII:C (U/mL)	vWF:Ag Plasma (U/mL)	vWF:Ag Platelet[c]	vWF:RCo Plasma (U/mL)	vWF:RCo Platelets[c]	XII (U/mL)
Father										
(4/1986)	253	15	92	D[b]	0.07	0.12	0.07	0.07	0.10	0.30
(11/1986)										
0'[a]	242	11	74	D[b]	0.17	0.22		0.13		0.48
30'		6			1.23	1.04		1.08		0.52
60'		5			1.08	1.04		0.94		0.52
2h					1.08	0.87		0.83		0.48
4h		6.5			0.72	0.87		0.60		0.52
Son										
(4/1986)	455	15	89	D[b]	0.07	0.12		0.07		0.24
(11/1986)	320	14	94	D[b]	0.11	0.12		0.07		0.06
Normal range	150-300	<9'	30-35		0.50-1.60	0.55-1.70	0.25-0.45	0.50-1.70	0.45-0.90	0.36-180

[a]Before (0') and after (30', 60', 2 or 4 h) administration of DDAVP.
[b]D: Ristocetin-induced platelet agglutination (RIPA) decreased.
[c]U/10^9 platelets/mL.

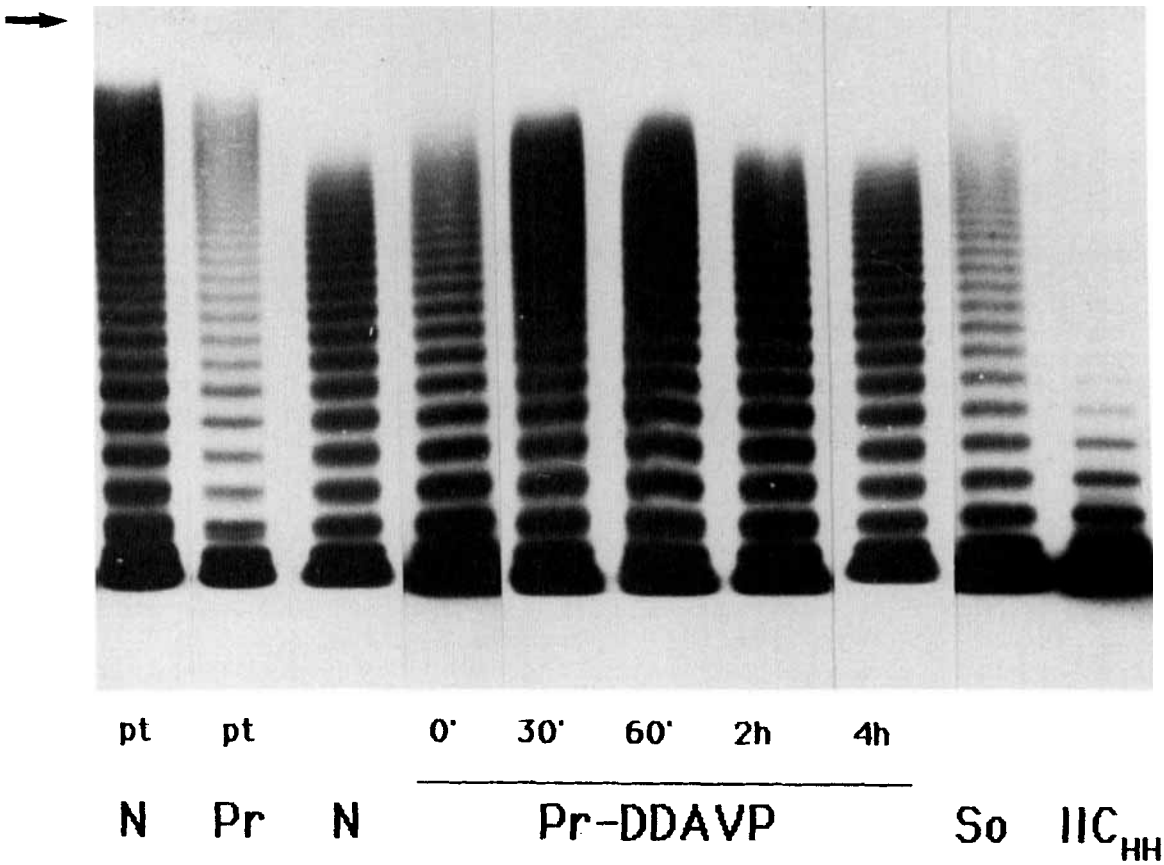

Fig. 1. SDS-agarose (1%, short gel) electrophoresis patterns of vWF in plasma and platelet lysate (pt) collected with inhibitors. From left to right: Normal individual (N). Propositus before (Pr) and after 30 min, 60 min, and 2 hr of DDAVP infusion (Pr-DDAVP, 0', 30', 60', 2h, respectively). Propositus's son (So) and a patient with homozygous or double heterozygous type IIC von Willebrand disease (IIC$_{HH}$). Each lane was actually run on the same gel at the same time; autoradiographs of different exposure times were obtained and the lanes cut and put together. Normal multimeric distribution is observed in the two patients, in plasma and platelet lysate as well, in contrast to the lack of large and intermediate multimers in vWF from the IIC patient. After DDAVP administration larger multimers than those seen in the resting conditions appear in plasma of the propositus.

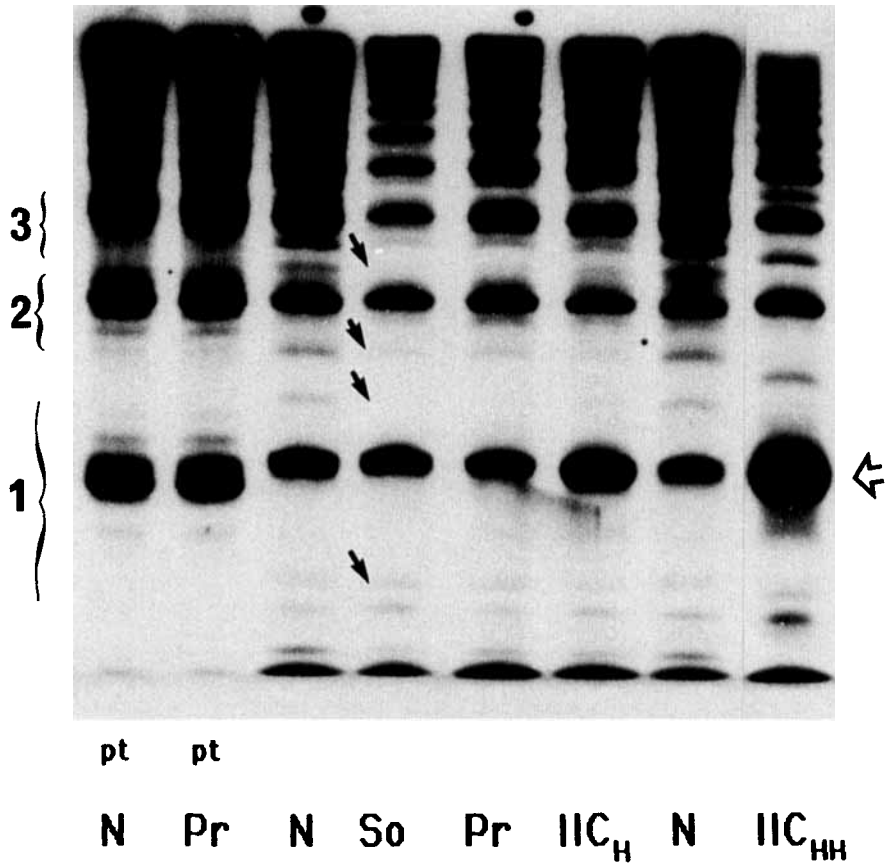

Fig. 2. SDS-agarose (2%, short gel) electrophoresis patterns of vWF in plasma and platelet lysate (pt) collected with inhibitors. From left to right: Normal individual (N). Propositus (Pr), propositus's son (So), patients with heterozygous and homozygous or double heterozygous IIC von Willebrand disease (IIC$_H$ and IIC$_{HH}$, respectively). Braces indicate the extension of first, second, and third multimers (1,2,3, respectively). The small solid arrows show the satellite bands of each triplet decreased. The white arrow shows the smallest oligomer increased, a hallmark of type IIC vWD. Normal multimeric distribution is observed in the two patients, in the platelet lysate of the Pr and S patients. For similar intensity of the central band, the satellite bands have a very decreased relative proportion, as is also seen in type IIC heterozygous patient. The latter becomes more evident in multimer 2. However, no marked increase in the smallest oligomer is present.

Multimeric analysis using low-resolution agarose gel (Fig. 1) showed the presence of all-size multimers in plasma and platelets of both patients. After DDAVP administration to the father, a response similar to that described in normal subjects was seen [9]. By using higher-resolution agarose gel electrophoresis (Figs. 2 and 3), vWF showed a normal pattern in platelets, whereas several distinct features were seen in every resolved plasma multimer. The main band of each multimer, though of normal intensity, showed a subtle but definite decreased mobility as compared with its counterpart in normal vWF. The slowest- and fastest-migrating bands of each plasma multimer had a very decreased relative proportion as also seen in the heterozygous IIC patient. Nevertheless, no marked increase in the smallest oligomer, a hallmark of type IIC vWD, was present in our patients (Figs. 2 and 3) [3]. Plasma vWF differed from platelet vWF in that the former did not show the fastest- and slowest-migrating bands seen in platelet multimers (Fig. 2).

After DDAVP infusion to the father, a transient increase in the relative proportion of the satellite bands of each multimer was observed, as we previously described in normal individuals [1], and in contrast to the heterozygous IIC patient (IIC$_H$) in whom vWF showed a much lesser increase in the satellite bands (Fig. 4).

The subunit analysis of vWF showed a normal distribution of the intact 225 kDa native subunit and of the 189, 175, and 140 kDa-derived fragments (Fig. 5). After DDAVP administration the decrease of the 225 kDa and 140 kDa bands as well as the increase of the 176 kDa band, usually seen in normal individuals, were also observed in the patient. However, an augmentation in the relative proportion of the 189 kDa band was noticeable in

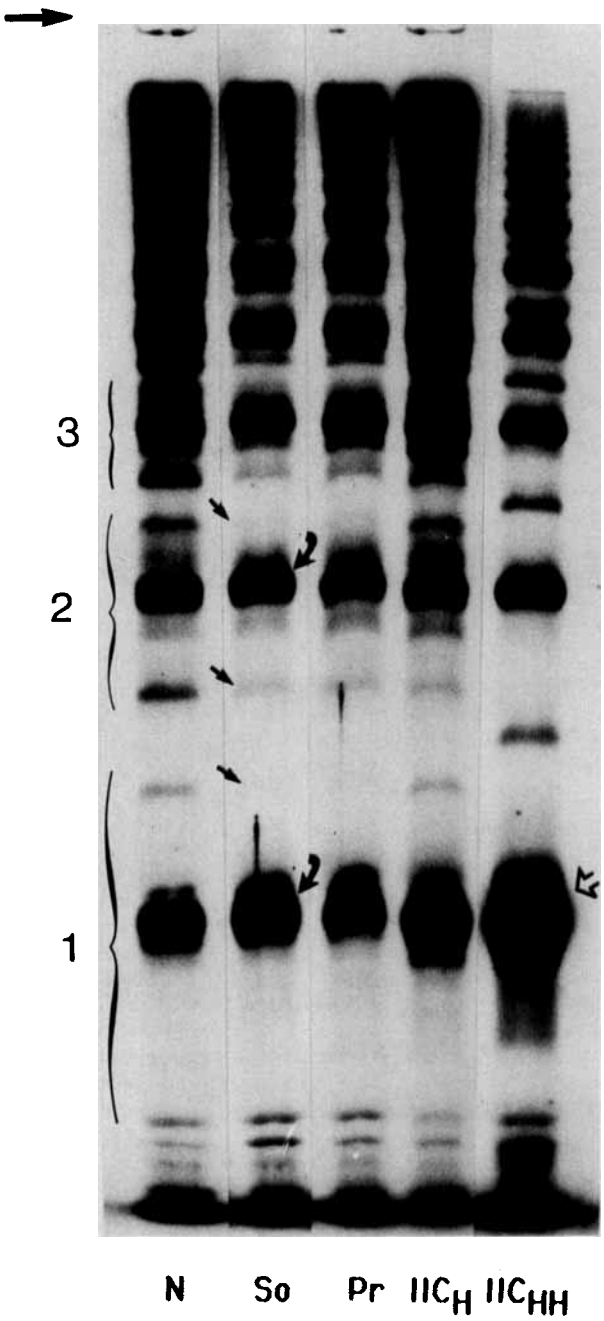

Fig. 3. SDS-agarose (2%, long gel) electrophoresis patterns of vWF in plasma collected with inhibitors. From left to right: Normal individual (N), propositus' son (So), propositus (Pr), patients with heterozygous and homozygous or double heterozygous IIC von Willebrand disease (IIC$_H$ and IIC$_{HH}$, respectively). Braces, small solid arrows, and the white arrow as in Figure 2. Each lane was actually run on the same gel at the same time; autoradiographs of different exposure times were obtained and the lanes cut and put together. The main band of each vWF of the propositus's and his son's multimers, though with an intensity similar to the normal vWF, shows a slightly decreased mobility as compared with its counterpart in normal vWF. For similar intensity of the central band, the satellite bands have a very decreased relative proportion, as is also seen in type IIC heterozygous patient. The latter becomes more evident in multimer 2. However, no marked increase in the smallest oligomer, a hallmark of type IIC vWD, is present.

the patient, in contrast to normal subjects in whom an opposite phenomenon occurs. In resting conditions, the heterozygous IIC patient (IIC$_H$) showed a partially decreased fragmentation of the 225 kDa subunit which increased to a lesser extent than in the normal subject when DDAVP was given (Fig. 5).

DISCUSSION

The family described here was characterized by a mild congenital hemorrhagic disorder transmitted, probably, as an autosomal dominant trait. Clincal symptoms included epistaxis, bleeding from the gums and after tonsillectomy or dental extractions, the latter requiring cryoprecipitate infusion on one occasion. The bleeding time was always prolonged. vWF:Ag and vWF:RCo were low. Because of these findings and the structural abnormality of vWF identified, the patients were diagnosed as having von Willebrand disease (vWD) associated with mild factor XII deficiency. As far as all the multimers are present in plasma and platelet vWF, the pattern corresponds to type I vWD. Since a certain degree of disproportion between the plasma levels of vWF:RCo and vWF:Ag is seen in resting conditions, the former being lower, and in accordance with the classification proposed by Ruggeri et al. [10], the patients should probably be included in the group "qualitative abnormalities of vWF and decreased platelet-vWF interaction."

Abnormalities of individual oligomers have been demonstrated in some form of type I vWD [11] as well as in several varieties of type II vWD [10–20]. The variant IC is characterized by an abnormal banding pattern of each vWF multimer, with decreased or missing satellite bands and alterations of the mobility (faster than in normal vWF). Treatment with DDAVP, although it corrected the BT, did not alter the structural abnormality. The patients described here also showed the presence of all the multimers in plasma and platelets (Fig. 1), as well as a decrease in the relative proportion of the satellite bands in each multimer (Fig. 2). But several distinct features were observed in both of them. The main band had a slightly decreased mobility when compared with its counterpart in normal plasma (Fig. 3). The infusion of DDAVP to the propositus was accompanied by a correction of the satellite bands (Fig. 4).

Type II vWD is defined by an absence of the larger multimers of vWF [10]. Type IIC shows a repeating doublet or single band as well as a marked increase of the smallest oligomer, a hallmark of this form of vWD. The heterozygous form of IIC vWD which has all the multimers in plasma and platelets in quite a similar fashion to our patients also shows a decreased intensity of the satellite bands of each multimer, but in contrast, has a marked increase of the smallest oligomer (Figs. 2 and 3) [1, 3, 12–14]. Furthermore, the heterozygous IIC patient

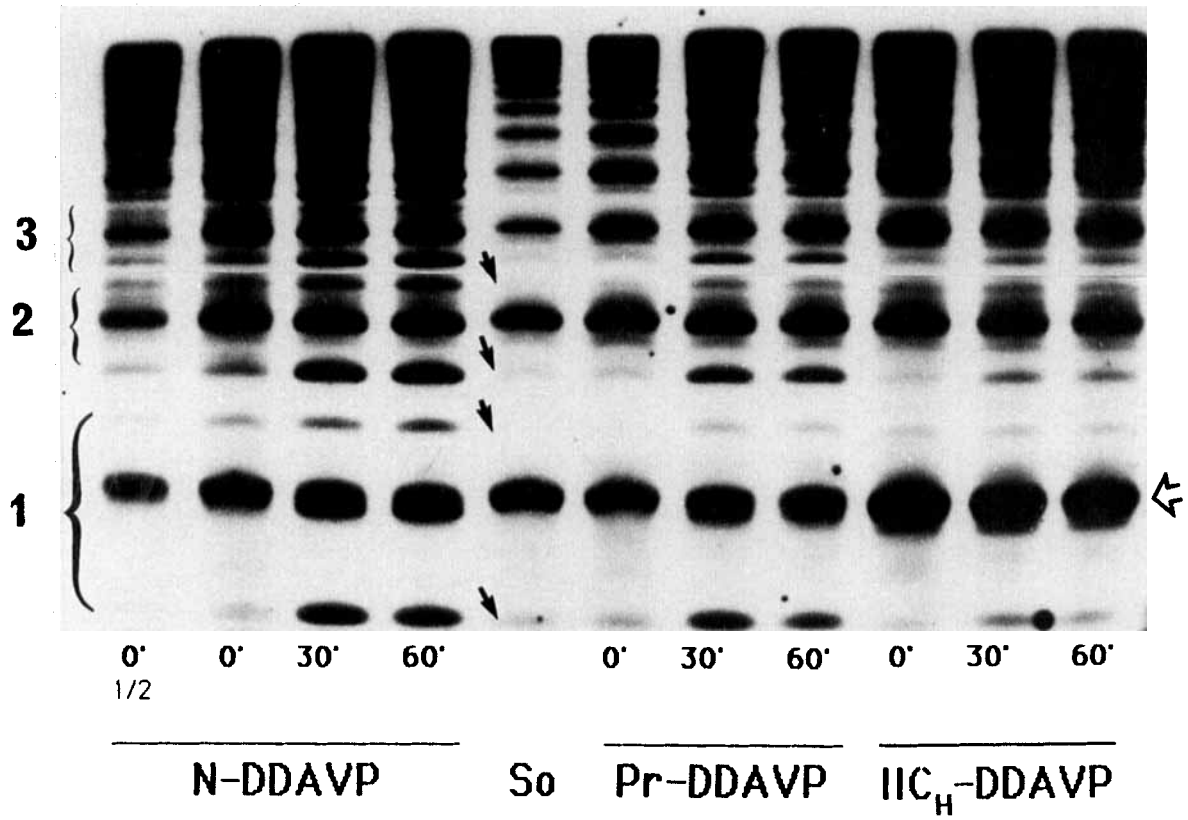

Fig. 4. SDS-agarose (2%, short gel) electrophoresis patterns of vWF in plasma collected with inhibitors. From left to right: Normal individual (N), Propositus's son (So), propositus (Pr), and a patient with heterozygous type IIC von Willebrand disease (IIC$_H$) before and after 30 and 60 min of DDAVP infusion (DDAVP: 0', 30', 60', respectively). Braces, small solid arrows, and the white arrow as in Figure 2. In order to better visualize the different bands of multimers 3 and larger that have a higher concentration than multimers 1 and 2, autoradiographs from two different exposure times of the same gel were obtained, then cut horizontally between multimers 2 and 3 and put together. After DDAVP infusion to a normal individual, a temporary increase in the satellite bands of each multimer is seen. The same phenomenon is observed in the propositus and IIC$_H$; this increase is, however, proportionally much less in the IIC$_H$ patient.

(IIC$_H$), presented in this study, after DDAVP administration, showed a much less pronounced augmentation in the relative proportion of the satellite bands (Fig. 4). On the contrary, type IIA vWD is characterized by pronounced satellite bands, particularly when blood is collected in the absence of protease inhibitors [10,15]. IID vWD [16,17] has a much more complex banding pattern in resting conditions. IIE vWF has only a clearly identifiable band but data on response to DDAVP were not reported [2]. IIF vWD also shows a normal platelet vWF, but no large and fewer intermediate forms are present in plasma, with a pattern of individual oligomers remarkably similar to that seen in platelets. After DDAVP infusion, the complex structure remained abnormal, although additional bands not clearly detected previously became evident after DDAVP [18]. IIG is characterized by the absence of only the slowest-migrating minor band in each oligomer and an almost normal pattern of platelet vWF analyzed in the presence of protease inhibitors [19]. Type IIH, instead of a triplet, presents a single broader central band with a minor, faster-moving satellite band, being particularly evident in the faster-moving multimers in plasma and platelet vWF. After DDAVP administration, there is no change in the abnormal structure [20].

As far as the subunit analysis of vWF is concerned, some fragmentation of vWF has been described in normal individuals in resting conditions [2]. Furthermore, we have reported a transient increase of proteolytic degradation of the native subunit of plasma vWF after DDAVP administration to normals [1]. The propositus described here showed abnormal behaviour in the proteolytic degradation of the vWF subunit after DDAVP administration. Thus the 225, 176, and 140 kDa bands experienced a variation similar to that seen in normal subjects, but in contrast, a relative increase of the relative proportion of the 189 kDa was observed in our patient. No subunit study was reported on type IC vWD. Type IIC

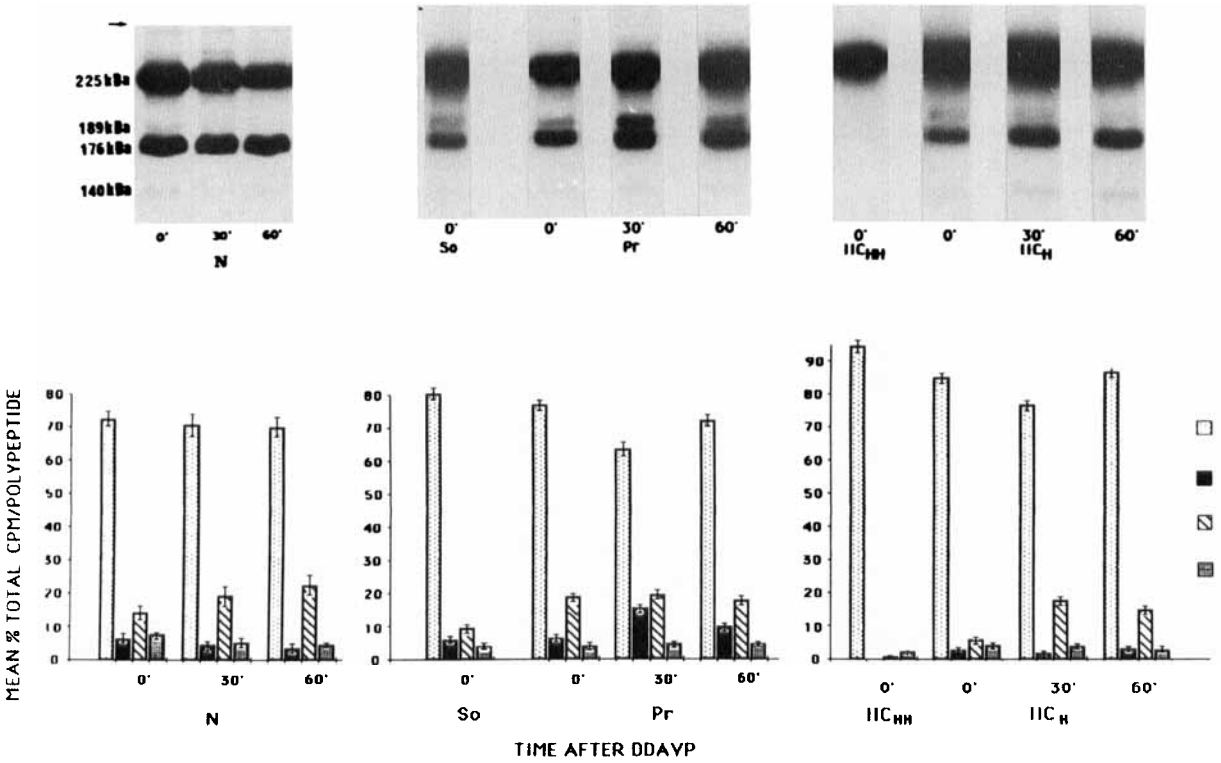

Fig. 5. Immunoblot autoradiography of reduced vWF and the relative proportion of counts present in each band. Upper panel: Reduced immunoisolated vWF following SDS-5%. PAGE and immunoblotting, before (0') and 30 and 60 min after DDAVP administration (30' and 60'): (from left to right): normal individual (N), propositus's son (So), propositus (Pr), and patients with homozygous or double heterozygous and heterozygous IIC von Willebrand disease (IIC$_{HH}$ and IIC$_{H}$). Lower panel: Relative proportion of counts in each of the bands shown in the upper panel, as determined by excising each band from nitrocellulose and measuring scintillation counts. The means and ranges were from three separate immunoisolations, gels, and immunoblots for each patient. The propositus and his son show a normal fragmentation pattern in resting conditions, in contrast to IIC$_{H}$ and particularly II$_{HH}$ patients, who show lesser or no fragmentation at all of the 225 kDa vWF subunit. After DDAVP infusion, the normal subject and all the patients show an increase in the relative proportion of the 176 kDa band as well as a decrease in the relative proportion of the 140 kDa fragment. The 189 kDa band is decreased in the normal individual and IIC$_{H}$ patient, but, in contrast, is increased in the propositus.

vWD is characterized by a subunit less proteolyzed than in normal vWF (Fig. 5) [2]. In resting conditions, the IIC heterozygous patient showed a very slight decrease of the subunit-derived fragments, and after DDAVP infusion to the heterozygous IIC patient, we observed a diminished fragmentation of the 225 kDa vWF subunit. This indicates that, in contrast with the propositus patient, the heterozygous IIC patient has a native vWF subunit more resistant to cleavage. Types IID and IIE vWD are also characterized by a decreased or absent proteolytic degradation of the vWF subunit [2]. A completely different situation has been observed in types IIA and IIB vWD in which the vWF subunit appears in plasma more cleaved even in a resting state [2,21]. Subunit analysis of vWF after DDAVP infusion had not been reported in patients with congenital vWD.

The multimeric abnormalities present in both patients described here could be explained by either a heterozygous pattern of a recessive type II vWD (as it occurs in type IIC in which the homozygous or doubly heterozygous patients have an absence of the largest multimers and the heterozygous patients have all the multimers in plasma but keep some abnormal features of the disease), or by a dominant one (as in type IC, with all the multimers present in plasma and platelets showing abnormalities of the intrinsic structure of the individual oligomers). In the former case, no homozygous or doubly heterozygous pattern was discovered; in this regard it would have been necessary to study other members of this family, which, unfortunately, was not possible. In any case, as already explained, these abnormalities were different from those previously described in other types of vWD.

In conclusion, this study describes a new variant form of vWD with aberrant structure of individual multimers with a certain degree of functional abnormality of vWF. This protein, despite being a less proteolyzed molecule than normal as suggested by the decrease of the satellite

bands observed in resting conditions, showed a clear proteolytic fragmentation following DDAVP infusion, though with an abnormal quantitative distribution of the subunit-derived fragments. To investigate the mechanism of this abnormal cleavage pattern, an epitope mapping of the vWF subunit [21] should be carried out in these patients.

This variant form is associated with a mild factor XII deficiency. The association of vWD and factor XII deficiency has been previously reported [22], but no structural defect has been described. More extensive family studies were not possible in this family to rule out conincidental concurrence of von Willebrand disease and heterozygous or homozygous factor XII deficiency. A possible role of factor XII deficiency on the structural abnormality of vWF remains to be investigated.

DDAVP infusion corrected the clinical symptoms and the BT, despite the abnormal fragmentation pattern seen after its administration, thus indicating once again that this drug is the first choice for treating bleeding diathesis in patients such as those described here.

In accordance with the presently accepted convention, we have designated this form of vWD type ID.

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