A Variant of von Willebrand’s Disease Characterized by Recessive Inheritance and Missing Triplet Structure of von Willebrand Factor Multimers

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A 10-yr.-old girl had bleeding symptoms of moderate severity; her mother and maternal aunt had milder bleeding symptoms, and other members of the kindred were asymptomatic. In the child, factor VIII coagulant activity (VIII:C) and von Willebrand factor antigen (vWF:Ag) were normal, ristocetin cofactor very low, and the bleeding time (BT) markedly prolonged. These values were normal in the rest of the kindred, but the mother and maternal aunt had prolonged BT and a high VIII:C/vWF:Ag ratio. Crossed immunoelectrophoresis (CIE) showed a vWF:Ag peak migrating more anodally in the propositus, two distinct peaks, one migrating anodally, in the father, paternal uncle, and grandmother, and normal peaks in the rest of the kindred. In the propositus, analysis of vWF multimers in plasma on 1.6% sodium dodecyl sulfate (SDS) agarose revealed that there were no larger multimers and there was a relative increase of the smallest multimer. This relative increase was also seen in her relatives with a double peak on CIE. Using gels of smaller porosity, each multimer of the propositus’s plasma consisted of a single band, instead of the repeating triplet seen in normal and von Willebrand’s disease variants types IIA and IIB. The abnormalities found in the propositus are tentatively interpreted as being due to double heterozygosity for two different genes. The defective gene carried by the father affects the triplet structure of vWF multimers, whereas a prolonged BT and a high VIII:C/vWF:Ag ratio are the only phenotypic expressions of the defective gene of the mother. The findings of aberrant triplet structure in congenital vWD strengthen the view that this structure is an intrinsic feature of the normal vWF molecule.

THE ANALYSIS of plasmas from patients with von Willebrand’s disease (vWD) by electrophoresis in sodium dodecyl sulfate (SDS) agarose gels and staining of von Willebrand factor antigen (vWF:Ag) with labeled affinity-purified antibodies1,2 has recently provided a new way to classify the disorder, based on the multimeric composition of von Willebrand factor (vWF). In the more common “classical” form of vWD, usually referred to as type I, the multimeric pattern is like that of normal plasma, but there is a lesser amount of each of the multimers. There are little or no slower-moving multimers of larger molecular weight in plasma of patients with “variant” forms of the disease, referred to as type II. Variants can be further subdivided into type IIA, in which the large and intermediate-size multimers are missing, and type IIB, in which only large multimers are missing.2 vWD type IIB is also peculiar in that platelet-rich plasma (PRP) from these subjects is hypersensitive to the aggregating agent ristocetin,1,4 which aggregates platelet-rich plasma (PRP) from types I and IIA patients poorly or not at all. Recently Ruggeri and Zimmerman5 have used an improved electrophoretic technique, based on a discontinuous buffer system and higher concentrations of agarose in the gels, to detect a more complex multimeric pattern. With their system, each multimer in normal and type I vWD plasmas is composed of three adjacent bands, instead of the single band seen with gels of larger porosity, and the central band of each “triplet” stains with the labeled antibody relatively more than the two adjacent bands. In type IIB vWD, the triplet structure does not differ from that of normal plasma, while type IIA vWD is characterized by a relative increase in staining of the adjacent bands of the triplet, approaching the central band in intensity.3 During a study utilizing this technique to analyze a large number of samples from vWD patients previously diagnosed in a national survey,6 we have identified a vWD variant characterized by an unusual mode of inheritance and an aberrant multimeric pattern with no triplet structure. This variant was directly compared with two variants reported by Armitage and Rizza,6 Hoyer,7 and Ruggeri et al.8 Our findings suggest that these cases are probably expressions of minor variations of the same genetic and molecular abnormalities.

MATERIALS AND METHODS

The propositus is a 10-yr-old girl from Rome with lifelong mild bleeding problems, including easy bruising, severe epistaxis, and excessive bleeding after superficial cuts and dental extractions. The mother has a history of easy bruising, epistaxis, and menorrhagia, and the maternal aunt had prolonged vaginal bleeding after each of three deliveries. Histories of the remaining members of the kindred...
were unremarkable. The patient and her relatives were studied on
three different occasions in Rome, and plasma samples and platelet
lysates were transported in dry ice to Milan. We have also studied
the propositus (A. M. IV 1) originally described by Armitage and
Rizza and subsequently restudied by Hoyer, his mother (R. M. III
3) and father (M. M. III 2). Frozen plasma samples were kindly
supplied by Dr. C. R. Rizza and transported to Milan in dry ice.
Plasma from the propositus (Pr) of the Swedish family reported by
Ruggeri et al. was restudied after storage at −70°C in our labora-
tory for 12 mo.

The methods for collection of blood and preparation of PRP and
platelet-poor plasma (PPP) have been published, as have the meth-
ods for ristocetin-induced platelet aggregation (RIPA) in PRP and
aggregation induced by adenosine diphosphate (ADP), epinephrine,
and collagen. Platelets were washed free of plasma constituents
and then lysed according to previously published procedures. Factor
VIII procoagulant activity (VIII:C) was assayed by a one-stage
clotting technique and concentrations, expressed in U/dl, were
referred to a pooled normal plasma calibrated against the First
International Reference Preparation for Factor VIII-Related Activ-
ities in Plasma (National Institute for Biological Standards and
Controls, London, UK). vWF antigen (vWF:Ag) was measured by
electroimmunoassay (EIA) and immunoradiometric assay
(IRMA), ristocetin cofactor activity was assayed with formalin-
fixed platelets. These measurements were expressed in U/dl, with
reference to pooled plasma calibrated against the International
Reference Preparation. Crossed immunoelectrophoresis (CIE) of
vWF:Ag was carried out as described except that 2% instead of 1%
agarose gels were employed. The multimeric composition of vWF
was analyzed by SDS thin-layer agarose electrophoresis, using the
discontinuous buffer system of Ruggeri and Zimmerman, differing
from their procedure in that electrophoresis was for 18 hr at a
constant current of 6 mA/gel (instead of 5–6 hr and 10–12.5
mA/gel). vWF multimers were identified autoradiographically by
exposing the gels to 125I-labeled affinity-purified anti-vWF antibod-
ies, prepared as described elsewhere from emu antiserum (kindly
provided by Dr. T. S. Zimmerman) or rabbit antiserum obtained as
described. Agarose gel concentrations were 1.6%, 2.0%, and
2.5%. 1-Deamino-(8-D-arginine)-vasopressin (DDAVP) was infused
i.v. into the propositus at a dose of 0.4 μg/kg, with blood samples
obtained and bleeding times (BT) measured (Simplate II, General
Diagnostics, Milano) before, during, and after the infusion,
accord-
ing to a protocol recently published.

RESULTS

The propositus of the Italian family and her relatives were studied
on three occasions and the median values of the laboratory findings are summarized in Table 1. The propositus (III 1) had normal VIII:C, normal
vWF:Ag by EIA, low vWF:Ag by IRMA; she also had no RIPA at the ristocetin concentrations tested (1.0, 1.5, and 2.0 mg/ml), very low ristocetin cofactor activity, and markedly prolonged BT. BT was also
prolonged in her mother (II 4) and maternal aunt (II 5), both of whom had normal VIII:C and borderline vWF:Ag by both IRMA and EIA; ristocetin cofactor activity was borderline in the mother but normal in the
maternal aunt. The paternal uncle (II 2) had slightly prolonged BT, but all the other values were normal for him and the rest of the family. Platelet aggregation with collagen, ADP, epinephrine, and ristocetin were
all normal for the entire family.

CIE of plasma vWF:AG disclosed one peak migrating more anodally than normally in the propositus; two distinct peaks, one migrating more anodally and one with normal mobility, in the father (II 1), paternal
uncle (II 2), and paternal grandmother (I 2); and a single normal peak in the remaining members of the kindred, including the mother and maternal aunt (Fig. 1). The three cases with the double vWF:Ag peaks had higher vWF:Ag by EIA than by IRMA (Table 1).

Analysis of vWF:Ag multimers in 1.6% agarose gels revealed that the propositus had no slower-moving (larger) multimers and that the fastest-moving (smaller-
Crossed immunoelectrophoresis patterns in agarose (2%) gel of von Willebrand factor antigen in plasma of the propositus (III 1, A), of the father of the propositus (II 1, B), and of two normal individuals (C and D). After electrophoresis in gel containing 125I-affinity-purified anti-vWF rabbit antibodies, an autoradiograph was made. III 1 plasma has a peak migrating more anodally than that of normal plasma; II 1 has a double peak, one of normal mobility, the other migrating more anodally than normal plasma.

The multimeric pattern was normal in the rest of the kindred. Gels of smaller porosity (2.0% and 2.5% agarose) disclosed a multimeric pattern in the propositus’s plasma that was strikingly different from those of normal, type IIA, or IIB vWD plasmas run in the same gel (Fig. 2). While these plasmas exhibited the typical multimeric pattern and triplet structures described by Ruggeri and Zimmerman, in the propositus, the two bands adjacent to the central predominant band of each triplet could not be detected, each multimer being thus composed of one band instead of three (Fig. 2). The fastest-moving multimer of the propositus stained more intensely than the corresponding multimer from normal and IIA or IIB vWD plasmas. Ahead of the fastest-moving multimer, corresponding to the anodal end of the gel, there were one or more additional bands staining with the antibody (Fig. 2). Such bands, which are also seen in plasmas from patients with severe vWD and unmeasurable vWF:Ag, are usually more prominent with the rabbit antibody than with the emu antibody. They are thought to be due to nonspecific binding of the labeled antibody to proteins other than vWF:Ag. The aberrant multimeric structure of the propositus’s plasma was also seen in her platelet lysate and did not change after DDAVP infusion (data not shown). In her father (Fig. 3), paternal uncle and grandmother, the triplet structure was intact but the fastest-moving multimer stained more intensely than in normal plasma. The mother (Fig. 3) and other maternal relatives had normal multimeric and triplet structures.

Figure 4 compares, in the same gel, at the same concentrations of vWF:Ag (~50 U/dl) and with the emu antibody used by Ruggeri et al., the multimeric pattern of the propositus’ plasma (III 1) and those of the propositus of Armitage (AM IV 1) and Ruggeri (Pr). The three propositi all lacked the largest multimers and had increased concentrations of the fastest-moving multimer and aberrant triplet structures. The latter differed, however, in that our propositus (III 1) showed only major bands without intervening bands, even after prolonged exposure of the gels, whereas the
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The main laboratory findings for the propositus—the presence of a more anodally migrating vWF:Ag peak on CIE and the absence of the larger multimers—are similar to those for previously reported vWD variants. However, the multimeric pattern in gels of smaller porosity were strikingly different for this case. The triplet structure seen in normal and vWD plasmas was not seen in the propositus, and only the central band of each triplet was present. An additional abnormality was the relative increase in the concentration of the fastest-moving multimer. Another peculiar feature of this case was the mode of inheritance. Types IIA and IIB vWD are transmitted with an autosomal locus in a dominant fashion, with the same abnormalities usually being found in affected antecedents and descendants. The genetics in our kindred differ from this simple pattern. The father and the paternal grandmother of the propositus had no histories of bleeding and normal laboratory findings, a double vWF:Ag peak on CIE, and an abnormal multimeric pattern characterized by an increased intensity of the central band of the fastest multimer, with intact triplet structure and larger multimers. In addition to those abnormalities, the paternal uncle also had a slightly prolonged BT. These findings indicate a dominantly transmitted qualitative abnormality of vWF on the paternal side of the family. Additional evidence in favor of this view is the observation that vWF:Ag was higher when measured by EIA then by IRMA, as previously reported for qualitative vWF defects. The genetic abnormality on the maternal side of the family is less clear. The discrepancy between low borderline levels of vWF:Ag and ristocetin cofactor (vWF-related measurements) and normal VIII:C (a factor-VIII-related measurement) in the mother and, less clearly, in the maternal aunt, is similar to that observed in the heterozygote state of recessively transmitted vWD, so that there might also be another qualitative abnormality of vWF that is only manifested by the BT prolongation. Combining the findings on the paternal and maternal side of the kindred, the findings in the propositus might be interpreted as indicating double heterozygositiy for two different genes. The father would carry one normal vWF gene and one defective gene, affecting the multimeric composition of
vWF:Ag, and the mother would carry one normal gene and one gene coding for a qualitatively and/or quantitatively abnormal vWF. The two gene abnormalities would then interact to result in the complex phenotype of the propositus.

Two previously reported cases may have had abnormalities similar to or identical with those reported here. Armitage and Rizza\(^6\) described a patient with recessively transmitted variant vWD, with his mother and five members on the maternal side of the family showing two distinct vWF:Ag peaks on CIE. Two of the individuals with the double peak also had prolonged bleeding times, like the paternal uncle of our propositus. Hoyer\(^7\) subsequently analyzed the plasmas of Armitage’s propositus and his mother by electrophoresis in an SDS agarose gel of relatively large porosity and found that the patient lacked the larger VWF:Ag multimers and had an increased concentration of the smallest multimer, and that his mother had normal amounts of the larger multimers and large amounts of the smallest multimer. It is conceivable that the prominent anodal component of the double VWF:Ag peak revealed by CIE in a number of relatives of both Armitage and Rizza’s patient and our case is related to the increase in the fastest multimer and that this component migrates more rapidly in the EIA, giving VWF:Ag levels higher than those measured by IRMA. Since Hoyer has not employed gels of low porosity, the triplet structure of the VWF:Ag multimers cannot be adjudged from his report. More recently, Ruggeri et al.\(^5\) have described a patient from Sweden in whom the normal triplet structure is replaced by a doublet with a major and a fainter band. Their case, which they call type II C vWD, appears to be similar to ours in terms of the main laboratory findings. The inheritance pattern cannot be compared, because the Swedish parents were not available, but the son of the propositus had an increased concentration of the smallest multimer. To establish whether these cases are really identical or not, the multimeric patterns of the three variants were reanalyzed and compared directly in our study. We basically found the same abnormalities in the propositus (no larger multimers, no triplet structure, increased fastest-moving multimer) and in one of the parents (defective triplet structure, increased fastest-moving multimer), whereas the remaining parent had a normal multimeric pattern and triplet structure. Some minor differences were also seen, in that the minor bands between the major bands in the patient of Ruggeri et al. were less evident in the case of Armitage and Rizza and not discernible at all in our own propositus. Until more is known about the significance of these abnormalities of the triplet structure, interpretations of the variations in the relative intensity of the intervening bands can only be speculative.

On the whole, these studies indicate that a classification of variant vWD based on the multimeric composition of vWF is more complex than had been thought. The findings of aberrant triplet structure in some types of congenital vWD strengthen the view that this structure is an intrinsic feature of the normal vWF molecule, rather than a technical artifact related to use of discontinuous buffer systems or gels of smaller porosity. The molecular nature of the triplet structure of vWF multimers in normal plasma and of its changes in some types of vWD remains to be elucidated.

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