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Type-3 von Willebrand's Disease in a Rhesus Monkey (*Macaca mulatta*)

Mary M. Patterson, DVM,[1,*] Lynn R. Jackson, DVM,[2,†] Marjory B. Brooks, DVM,[3] and James L. Catalfamo, PhD[3]

Severe type-3 von Willebrand's disease (vWD) was diagnosed in a young male rhesus monkey that had excessive bleeding from minor wounds. Plasma samples from the monkey had no detectable quantitative or functional von Willebrand factor (vWF), low Factor-VIII coagulant activity, and moderate prolongation of activated partial thromboplastin time. Testing of the affected monkey's extended family revealed a likely hereditary basis for the vWD, in that the sire and a paternal half-sister had markedly reduced plasma vWF concentration. Fresh whole blood was transfused to control frequent bleeding episodes throughout the monkey's life. Although vWD is the most common inherited bleeding disorder in humans and dogs, this is the first report of vWD in a nonhuman primate.

Von Willebrand's disease (vWD) is caused by a quantitative or qualitative defect in von Willebrand factor (vWF) protein (1-3). Inherited vWD is the most common bleeding disorder in humans and dogs, and has been reported in a number of other species such as pigs, mice, horses, and cattle (4-6). Nevertheless, vWD has not been described previously in a nonhuman primate.

Case Report

An eight-month-old, male rhesus macaque (*Macaca mulatta*) was observed to be less active than usual and to have blood on its hands and feet. Two months earlier, the monkey had been transferred from its birth harem group at the New England Regional Primate Research Center (NERPRC) to peer-group housing at the same facility. The NERPRC animal care program is fully approved by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Ketamine hydrochloride (approx. 10 mg/kg of body weight) was administered intramuscularly to the 1.6-kg monkey to facilitate a physical examination. Small abrasions and puncture wounds were apparent on the monkey's extremities and face. In addition, the monkey had pale mucous membranes, slow capillary refill time (> 2 sec), tachycardia (heart rate, 240 beats per minute), and miotic pupils. The remainder of the examination was unremarkable. A spun hematocrit (Hct) was 23%. Supportive therapy for shock and anemia was initiated and consisted of lactated Ringer's solution (100 ml, i.v.), dexamethasone (1.6 mg, i.v.), iron dextran (25 mg, i.m.), and oral vitamin supplementation. The monkey was singly housed in the clinic to allow frequent monitoring.

Over the next three days, the monkey became more alert and active, with a good appetite and normal feces. However, intermittent bleeding from minute lacerations was observed on both ear pinnae. Because the hemorrhage appeared excessive in volume and duration for the severity of the wounds, a complete blood count (CBC) and coagulation screening tests were performed. The results of the CBC (given as the patient's value followed by NERPRC reference range; Model 9018, Seroma Baker Diagnostics, Allentown, Pa.) indicated regenerative anemia and mild thrombocytopenia: 2.62 (4.98 to 6.42) × 10^6 RBC/μl; Hct 20.6 (37.2 to 47.1) %; hemoglobin (Hb) concentration, 6.9 (11.7 to 14.7) g/dl; mean cell volume (MCV), 79.0 (69.0 to 79.0) fl; mean cell Hb (MCH), 26.3 (21.7 to 24.7) pg; mean cell Hb concentration (MCHC), 33.5 (30.2 to 32.8) %; 114 (190 to 536) × 10^3 platelets (Plt)/μl; 10.5 (3.4 to 11.2) × 10^3 WBC/μl; 2+ polychromasia; and 2+ anisocytosis. Activated partial thromboplastin time (APTT) was 33.3 sec, and prothrombin time (PT) was 10.5 sec. These values were considered to be within normal limits on the basis of published values for other species of nonhuman primates (7); albeit no in-house ranges for rhesus macaques were available from the commercial diagnostic laboratory.

The affected monkey was transfused with 35 ml of fresh whole blood drawn into citrate-phosphate-dextrose-adenine anticoagulant from an unrelated adult rhesus monkey. One week later, results of a second CBC indicated resolution of the anemia and thrombocytopenia, with a Hct of 40.3% and Plt count of 645 × 10^3/μl. The monkey's pinnal wounds had healed, and it was returned to the peer-group gang cage.

A second bleeding episode occurred 12 days later and was characterized by marked hemorrhage from multiple minor abrasions on the face, limbs, and scrotum. Epistaxis and a grade-II/VI systolic murmur were additional clinical findings. Laboratory evaluation included a second coagulation screen and serum biochemical analysis; results of both were interpreted as being within normal limits. As before, the CBC revealed anemia (Hct, 22.3%) with mild thrombocytopenia (178 × 10^3 Plt/μl). The monkey's clinical signs resolved following a whole blood transfusion and bandaging of the intravenous catheter site and a wound on the left hand. Henceforth the monkey was maintained in a cage by itself to prevent trauma inflicted by cagemates.

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[1]Division of Comparative Medicine, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, [2]New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772, and [3]Comparative Coagulation Section, Department of Population Medicine and Diagnostic Science, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853.
[†]Present address: Biogen, Inc., 14 Cambridge Center, Cambridge, Massachusetts 02142.
[*]Corresponding author.

Table 1. Results of coagulation and von Willebrand factor assays in rhesus monkeys

	APTT (sec)[a]	PT (sec)	FVIII:C (%)	FIX:C (%)	FXI:C (%)	vWF:Ag (%)	vWF:RCof (%)
Affected male	33.9	12.6	14	74	136	< 0.1	0
Dam	22.2	12.8	144	102	139	68	60
Actual sire	23.8	13.0	49	70	136	24	15
Suspect sire	22.9	12.7	142	84	140	120	124
Submitted control	20.2	12.3	191	89	147	139	134
Pooled rhesus plasma	21.3	11.3	100	100	100	100	100

[a]APTT = activated partial thromboplastin time, PT = prothrombin time, FVIII:C = factor VIII coagulant activity, FIX:C = factor IX coagulant activity, FXI:C = factor XI coagulant activity, vWF:Ag = von Willebrand factor antigen concentration, vWF:RCof = ristocetin cofactor activity of von Willebrand factor.

The nature and severity of the monkey's bleeding diathesis were considered unlikely to be caused by mild thrombocytopenia. Therefore, more comprehensive hemostasis testing was performed after the monkey's condition had stabilized, with no bleeding episodes or transfusions, for a period of four weeks. Blood samples were drawn directly into evacuated tubes containing citrate (1 part 3.8% sodium citrate to 9 parts blood) and were centrifuged, then the supernatant plasma was shipped on dry ice to the Comparative Coagulation Section laboratory at Cornell University. A second citrate-anticoagulated plasma sample was submitted a few weeks later for corroboration. At that time, several additional monkeys also were tested to investigate the possibility of a hereditary basis for the condition. Plasma from an unrelated, healthy rhesus monkey was included with each shipment as a control for sample collection and shipping conditions. Coagulation assays included APTT (8) (Dade Actin FS, Baxter Diagnostics, Edison, N.J.), PT (8) (Thromboplastin L, Pacific Hemostasis, Huntersville, N.C.), and specific coagulant activities of Factor VIII (FVIII:C), Factor IX (FIX:C), and Factor XI (FXI:C). Factor activity assays were performed using a modified one-stage APTT (9) technique and human substrate-deficient plasmas (George King Bio-medical, Inc., Overland Park, Kans.). All clotting time tests were performed using a photo-optical clot detection instrument (Coag-A-Mate, Organon Teknika, Durham, N.C.). Quantitative and functional analyses of plasma vWF consisted of vWF concentration (von Willebrand factor antigen [vWF:Ag]) and vWF ristocetin cofactor activity (vWF:RCof), respectively. The former was measured using an ELISA with polyclonal anti-human vWF antibodies (10). Ristocetin cofactor activity was determined by measuring the agglutination of formalin-fixed human platelets (Lyophilized Platelets, Biodata, Horsham, Pa.) in dilutions of test plasma (11). Results of vWF:Ag and all factor activity assays were reported as the percentage of a pooled monkey plasma standard (prepared from six clinically normal rhesus monkeys) having an assigned value of 100%. Structural analyses of vWF protein were performed by immunoelectrophoresis and immunoblot (western blot) analysis (12) with detection of vWF multimeric forms using a primary rabbit anti-human vWF antibody (von Willebrand Factor, Rabbit anti-human, Accurate Chemical Corp., Westbury, N.Y.).

Coagulation and vWF analyses for the affected monkey (Table 1) revealed the following abnormalities: moderate prolongation of APTT (33.9 sec, compared with 21.3 sec for the pooled rhesus sample), low Factor VIII:C (14%) activity, and no detectable vWF:Ag (< 0.1%) or vWF:Rcof (0%). These abnormalities were present at both sample time points. Results of the tests for the dam's Factor VIII:C, vWF:Ag, and vWF:Rcof were > 50% of the pooled rhesus control value (Table 1). While a reference range for these parameters in rhesus monkeys has not been established, 50% is accepted as an appropriate cutoff for normal subjects in the characterization of vWD in human beings, horses, and cattle (3, 5, 6).

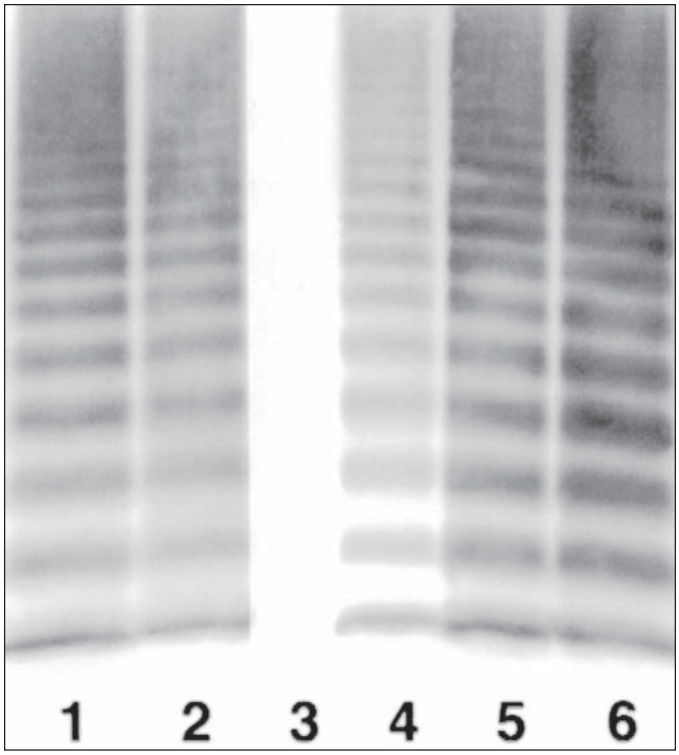

Figure 1. Immunoblot (western blot) prepared from agarose gel electrophoresis of plasma von Willebrand factor from rhesus monkeys. Lanes: 1 = pooled normal rhesus plasma control (vWF:Ag = 100%); 2 = dam of affected male (vWF:Ag = 68%); 3 = affected male (vWF:Ag < 0.1%); 4 = sire of affected male (vWF:Ag = 24%); 5 = suspect sire (vWF:Ag = 120%); and 6 = submitted control rhesus monkey (vWF:Ag = 139%). The origin of the separating gel is at the top, with smallest vWF molecular weight multimers at the bottom of each lane.

Two potential sires in the harem breeding group were tested. The dominant male's plasma ("Suspect sire" in Table 1) had normal vWF:Ag, vWF:RCof, and FVIII:C values, whereas a lower ranking male ("Actual sire" in Table 1) had low values. Subsequent DNA testing that involved polymerase chain reaction analysis of short tandem repeat markers (13), performed on EDTA-preserved whole blood, was used to confirm paternity of the latter male. Electrophoretic analyses of vWF (Fig. 1) demonstrated no hybridization of anti-vWF antibodies with the affected male's sample. In contrast, the other monkeys that were tested had a full-size distribution of vWF multimeric forms.

Discussion

The affected monkey's clinical signs and complete lack of de-

tectable vWF were compatible with a diagnosis of type-3 vWD (3). Von Willebrand factor is required for platelet adhesion to injured vessel walls and subsequent thrombus formation. After its synthesis by endothelial cells and megakaryocytes, the mature vWF glycoprotein is found in the subendothelium and plasma, and consists of an array of multimeric forms. These multimers are made up of identical subunits joined by disulfide bonds. In type-3 vWD, the most clinically severe and rare variant, affected patients lack any of the vWF multimeric forms. Type-1 and type-2 vWD are typically mild to moderate bleeding diatheses (1-3). The classification of type-1 vWD is characterized by low plasma vWF concentration, with all multimeric forms present. Plasma vWF concentration is low in patients with type-2 vWD, and the highest molecular weight vWF multimers are absent. Besides supporting platelet adhesion, plasma vWF binds noncovalently to coagulation Factor VIII, thereby preventing its rapid clearance from the circulation and localizing the coagulation cofactor to the site of vessel injury. The marked reduction in FVIII:C measured in the affected monkey's plasma is typical of type-3 vWD in human patients, and can be attributed to the absence of plasma vWF with resultant short Factor VIII plasma half-life (2). The affected monkey's long clotting time in the APTT assay can be explained by low FVIII:C activity, because the APTT screening test is sensitive to deficiencies of Factor VIII.

Type-3 vWD is typically inherited as an autosomal recessive trait. In most type-3 kindreds, heterozygous carriers of a vWF mutant allele are clinically normal, although their plasma vWF concentration is often low (1, 2, 14). When two candidate sires of the affected male were screened for vWD, one of them had decreased values for FVIII:C, vWF:Ag, and vWF:Rcof. These data were supportive of vWD carrier status, and paternity testing ultimately resolved the issue. Clinical bleeding problems were never reported for the sire.

The dam had somewhat reduced vWF:Ag (68%) and vWF:RCof (60%) values relative to the pooled rhesus plasma standard. Although these values were sufficient to exclude a bleeding tendency in the dam due to vWF deficiency, its carrier status for the vWD trait could not be assigned on the basis of these data. A complicating factor was that this female was in late pregnancy when the sample was collected, and pregnancy is known to increase plasma vWF:Ag concentration in women and other species (15, 16). The dam was retested immediately after another pregnancy; however, problems with sample quality precluded a more definitive estimation of the baseline vWF:Ag concentration.

A pedigree chart with vWF:Ag values for the extended family of the affected male is shown in Fig. 2. Along with the sire and dam described previously, four additional relatives were located and tested. Two maternal half-sisters and the paternal grandsire had normal vWF:Ag activity. A paternal half-sister, however, had very low vWF:Ag activity (27%). The findings of low vWF:Ag activity in this paternal half-sibling and the sire suggest that they are heterozygous carriers of a vWF gene mutation. In the case presented here, the condition appears derived from the paternal line, but whether the maternal line has contributed a defective allele remains unknown. Compound heterozygosity, with different vWF mutations transmitted from each parent, could also be the cause of type-3 vWD in offspring of matings between unrelated individuals. Experimental matings and molecular analyses of the vWF gene would be necessary to clarify the mode of inheritance and define the nature of the causative vWF mutation(s) in this family of monkeys.

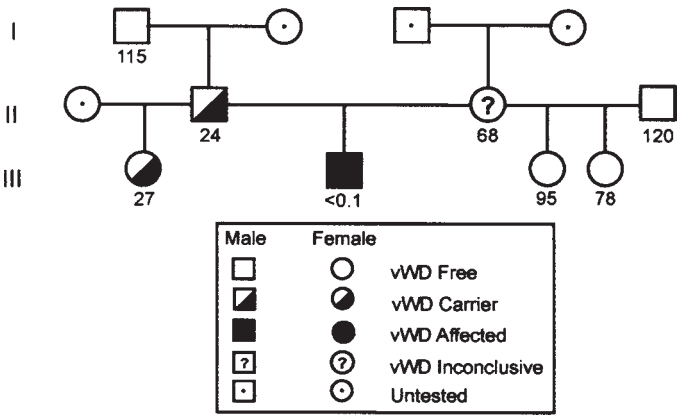

Figure 2. Pedigree chart of related rhesus monkeys. Roman numerals at left represent generation. Numbers under symbols are plasma vWF antigen activity (vWF:Ag %).

The affected monkey continued to have episodes of bleeding that required intensive medical care. Additional clinical signs included hematomas over the carpal joints and melena; the latter may have resulted from incidents of epistaxis. Because fresh whole blood was efficacious and readily available from donors, the monkey was not transfused with cryoprecipitate, the treatment of choice for supplying active vWF to human vWD patients (17). Despite numerous transfusions from multiple donor monkeys at the NERPRC, immune-related reactions to red blood cell or other antigens were never manifested clinically. The frequency of transfusions fluctuated throughout the monkey's life, and diminished once single housing reduced the risk of injury. Monthly transfusions were common at one year of age, but shortly thereafter there was a nine-month hiatus in the medical history with no bleeding crisis warranting transfusion. Prolonged intervals between apparent spontaneous bleeding episodes are also commonly reported for human type-3 vWD patients (18). Not surprisingly, numerous instances of hemorrhage developed at tooth eruption sites in the affected monkey. These necessitated frequent blood transfusions, and humane concerns led to a decision to euthanize the monkey at four years of age.

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