RAPID COMMUNICATION

An Autosomal Dominant, Qualitative Platelet Disorder Associated With Multimerin Deficiency, Abnormalities in Platelet Factor V, Thrombospondin, von Willebrand Factor, and Fibrinogen and an Epinephrine Aggregation Defect

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Multimerin is a massive soluble, multimeric protein found in platelets and endothelial cells. Recent studies identified multimerin as a specific coagulation factor V binding protein, complexed with platelet, but not plasma, factor V. These findings led us to investigate individuals with inherited factor V deficiencies for possible multimerin abnormalities. Platelet proteins were evaluated using immunosassays, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, immunoblotting, immunoprecipitation, and direct binding studies. Patients with factor V Quebec, a disorder with abnormal platelet factor V, had a quantitative deficiency in multimerin (n = 11 tested; mean, 12.5%; range, 5% to 27% of the normal pool; normal range, 45% to 214%) with a normal multimer pattern. Quantitative and qualitative abnormalities were detected in their platelet factor V. An unrelated patient who was deficient in platelet and plasma factor V had normal platelet multimerin. The levels of platelet β-thromboglobulin, von Willebrand factor, thrombospondin, and fibrinogen antigen were normal in the factor V Quebec patients. However, proteins with abnormal mobility were detected in their platelet lysate and releasate, and their platelet thrombospondin, von Willebrand factor, and fibrinogen showed evidence of proteolytic degradation. Platelet counts of the factor V Quebec patients ranged from mildly thrombocytopenic to low normal (mean, 159 × 10^9/L; range, 104 to 198 × 10^9/L). In addition, their platelets failed to aggregate in response to 6 to 10 μmol/L epinephrine despite normal numbers of platelet α2-adrenergic receptors. These data indicate that patients with factor V Quebec have an inherited bleeding disorder distinct from other platelet disorders and associated with multiple abnormalities, including multimerin deficiency, abnormal platelet factor V, thrombospondin, von Willebrand factor, and fibrinogen, and an epinephrine aggregation defect.

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bleeding disorder distinct from other qualitative platelet disorders that is associated with multimerin deficiency.

MATERIALS AND METHODS

Patients

All studies were performed with institutional ethics approval and patient consent. Clinical histories were obtained by interviewing family members and reviewing medical records. Initial studies were performed on five representative members of the factor V Quebec kindred (Fig 1): three individuals with bleeding symptoms (V 22, V 23, and VI 10) and two individuals without bleeding problems (VI 8 and VI 9). Samples obtained from additional asymptomatic (IV 4-1 and IV 4-2) and symptomatic (IV 5, IV 6, IV 17, IV 20, IV 21, IV 23, V 32, and V 33) family members were used for subsequent studies. In addition, healthy controls, an unrelated patient with factor V deficiency (2% plasma factor V coagulant activity), and unrelated patients undergoing investigations for bleeding problems (including patients with von Willebrand’s disease and patients with thrombocytopenia due to inherited, immune, and nonimmune causes) were evaluated. Samples from healthy controls were processed in parallel with all patient samples.

Laboratory Investigations

Preparation of platelet lysates. Whole blood (30 mL) was collected into acid-citrate-dextrose (ACD; 5 mL) containing platelet activation inhibitors and protease inhibitors (2 μmol/L prostaglandin E, [PGE], 50 μg/mL leupeptin, 1 mg/mL iodoacetamide, 0.25 mmol/L phenylmethylsulfonyl fluoride [PMSF], and 1 μg/mL aprotonin). Platelet-rich plasma (PRP) was harvested and the platelets were washed twice using 10 mL volumes of phosphate-buffered saline, pH 7.4, containing 5 mmol/L EDTA and 1 mmol/L theophylline. Platelets (1 × 10^9/mL) were lysed as described using 0.5% Triton X-100 or 1% sodium dodecyl sulfate (SDS) and protease inhibitors (5 mmol/L EDTA, 50 μg/mL leupeptin, 0.25 mmol/L PMSF, 1 mg/mL iodoacetamide, or 5 mmol/L N-ethylmaleimide) and stored in aliquots at −70°C before analysis. For some studies, the platelet pellet from PRP (containing inhibitors) was immediately solubilized and frozen (−70°C) for subsequent analyses. Triton X-100 lysates were centrifuged (15,000g for 10 minutes) to remove insoluble proteins before quantitative analyses.

Glycoprotein analyses. Immunoblot analyses of platelet proteins were performed as described using 2 to 50 μL of lysate, reduced SDS-polyacrylamide gel electrophoresis (SDS-PAGE) or nonreduced multimer gels, transblotted onto nitrocellulose, and reacted with monoclonal and polyclonal antisera, followed by detection using alkaline phosphatase or peroxidase-conjugated secondary antibodies and nitroblue tetrazolium chloride 5-bromo-4-chloro-indolyl-phosphate (NBT-BCIP) or Renaissance Western Blot Reagent chemiluminescence (Dupont NEN, Boston, MA) substrates. For some studies, platelets were labeled with biotin, solubilized, and used for immunoprecipitation and SDS-PAGE analyses, as described. Antibodies used for investigations included monoclonal (JS-1) and polyclonal antisera against multimerin, monoclonal antithrombom...

Fig 1. Factor V Quebec family pedigree. The numbers inside symbols indicate more than one individual. Two of the four family members designated by symbol IV 4 were studied; these individuals (IV 4-1 and IV 4-2) were asymptomatic.
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bospodin (CH-1), monoclonal anti-GPβδ2 (Raj-1), monoclonal anti-GPIX (Beb-1), and polyclonal antihuman factor V (horse antisera [Immunologic Technologies, Essex, VT]), rabbit antisera [Alexis Biochemicals], and sheep antisera [Affinity Biologicals, Hamilton, Ontario, Canada], polyclonal antihuman von Willebrand factor (Dako, Carpinteria, CA), monoclonal antithrombospondin (Alexis Biochemicals), and polyclonal antihuman fibrinogen (rabbit antisera [Behring Diagnostics Inc, Westwood, MA] and goat antisera [Organon Teknika, Scarborough, Ontario, Canada]).

Platelet multimerin, factor V, von Willebrand factor, fibrinogen, and thrombospondin were quantitated using enzyme-linked immunosorbent assay (ELISA). Standard curves were prepared using pooled platelet lysate from 20 healthy individuals. Because of differences in the molecular weights of platelet and plasma factor V, pooled platelet lysate was also used as the standard for the platelet factor V ELISA assay. Determinations of platelet multimerin, factor V, thrombospondin, and fibrinogen antigen levels were performed as follows: FS-1 (monoclonal antimitrimerin), horse antihuman factor V, CH-1 (monoclonal antithrombospondin), or goat antihuman fibrinogen, were used to coat Immulon II microtitre plates (Dynatech Laboratories Inc, Alexandria, VA; 1 µg IgG/well, in carbonate buffer pH 9.6, overnight incubation). Plates were washed (triplicate) using Tris-buffered saline (20 mmol/L Tris-HCl, 150 mmol/L NaCl, pH 7.5, with 0.02% Tween 20) between incubations (1 hour at room temperature). Wells were blocked (2% bovine serum albumin in phosphate-buffered saline) and then incubated with dilutions of platelet lysate (in lysis buffer containing 0.5% Triton X-100). The bound multimerin was detected using rabbit antimitrimerin (1:1,000 dilution), followed by alkaline-phosphatase-conjugated goat anti-rabbit IgG (BioCan Scientific Inc, Mississauga, Ontario, Canada; 1:1,000 dilution containing 10 μg/mL normal mouse IgG [Organon Teknika]) and p-nitrophenyl phosphate substrate tablets (Sigma, St Louis, MO; OD 405 recorded). For the thrombospondin and fibrinogen assays, rabbit antithrombospondin (1:1,000 dilution) and rabbit antifibrinogen (1:2,500 dilution) were used. Factor V was detected using peroxidase-conjugated sheep antihuman factor V (10 µg/mL) and 1-Step Turbo TMB-ELISA (Pierce, Rockford, IL; reactions terminated at 20 minutes using 0.5 N H2SO4, OD 450 measured). Dilutions of plasma and platelet lysate yielded parallel factor V antigen curves and no factor V antigen was detected in factor V-deficient plasma (Dade International, Mississauga, Ontario, Canada). All samples were assayed in triplicate at dilutions ranging from neat to 1:4,000, and all abnormal results were confirmed in repeat assays.

Normal ranges for platelet glycoprotein quantitations were determined from lysates prepared from healthy donors. The normal range for platelet multimerin antigen levels was calculated using log transformed data to normalize the distribution. Platelet multimerin antigen levels in lysates prepared from washed platelets or from platelets pelleted from PRP gave identical results in the ELISA.

Analyses of platelet factor V antigen levels were performed using additional factor V ELISA, and aliquots of the same samples were also analyzed by Dr K.G. Mann's laboratory (University of Vermont, Burlington, VT), using a competitive binding radioimmunoassay (RIA) and purified plasma factor V as the standard.

Studies of platelet α2-adrenergic receptor number and function. Platelet epinephrine aggregation studies were performed using 6, 10, and 40 µmol/L final epinephrine concentrations. For thrombocytopenic patients with PRP platelet counts less than 250 × 10^9/L, testing was performed in parallel with a sample from a healthy control diluted to the same platelet count.

Platelet α2-adrenergic receptor numbers were quantitated by direct binding with epinephrine receptor antagonist yohimbine, using modifications of methods previously described. Briefly, whole blood was collected into ACD, and the platelets were washed twice, using calcium-albumin–free Tyrode's buffer, pH 6.2, containing 1 mmol/L theophylline, and resuspended in Tris-saline EDTA (20 mmol/L Tris, 130 mmol/L NaCl, 5 mmol/L EDTA, pH 7.4). Platelets (100 µL of 1 to 3 × 10^9/mL) were added to microtiter wells containing [3H]yohimbine (Dupont NEN; 20 µL Tris-saline EDTA; 1 to 20 mmol/L final concentrations). After 30 minutes of incubation at 37°C, the platelets were harvested onto glass fiber filters (Cambridge Technology Inc, Watertown, MA) using a PHD cell harvester (Cambridge Technology Inc). The bound counts were separated from unbound by filling and aspirating the microtiter wells 15 times with Tris-saline EDTA. The filter disks were placed in vials containing 6 mL of ACS scintillation cocktail (Amersham Canada Ltd, Oakville, Ontario, Canada) and counted. Specific binding was calculated by subtracting the counts bound in the presence of 100 µmol/L cold yohimbine (Sigma). Receptor numbers and binding affinities were determined by Scatchard analysis.54

RESULTS

Family History

Figure 1 shows the pedigree of the factor V Quebec family, the individuals studied, and several individuals previously tested for platelet aggregation defects. The bleeding disorder in the family is inherited as an autosomal dominant trait, and the symptomatic individuals have suffered from episodes of mucocutaneous bleeding and delayed onset (12 to 24 hours), moderate-severe bleeding after trauma or surgery. Less common bleeding manifestations have included spontaneous hemarthroses, spontaneous intracranial hemorrhage, and rarely, umbilical cord bleeding. Figure 2 shows an oral hematoma that developed in V 22 with the eruption of a wisdom tooth and an extensive hematoma that developed after a snowmobile ride. Hemarthroses have occurred in V 22 (Fig 2) and in many affected family members after trauma, but spontaneous hemarthroses have also occurred. Three family members have suffered spontaneous intracranial hemorrhage. Many of the affected individuals have received large quantities of blood products (fresh frozen plasma, cryoprecipitate, and platelet concentrates) without control of their bleeding. Reduced bleeding has been observed with fibrinolytic inhibitors, during pregnancy, and with oral contraceptive use.

The platelet counts of asymptomatic family members evaluated were normal (n = 4; range, 217 to 289 × 10^9/L). At the time these investigations were performed, the platelet counts in the symptomatic family members (n = 11; mean, 159 × 10^9/L; range, 104 to 198 × 10^9/L) were significantly different from the healthy controls (n = 20; mean, 239 × 10^9/L; range, 148 to 349 × 10^9/L; P < .01). Some of the patients (V 22 and V 23) have had documented chronic thrombocytopenia, with platelet counts as low as 80 × 10^9/L. Two patients with normal platelet counts (IV 21 and V 33) were thrombocytopenic on subsequent evaluations. The mean platelet volumes of all symptomatic family members studied were normal.

Previous coagulation investigations on these patients indicated bleeding times that ranged from normal to mildly prolonged, and their plasma factor V coagulant activities have been either mildly reduced or normal. Normal tests of heamostasis included plasma coagulation factors I, II, VII, VIII, IX, X, XI, XII, and XIII; PAI-1; von Willebrand factor (antigen, ristocetin cofactor activity, and multimers); whole blood and euglobulin clot lysis times; platelet morphology (light
and electron microscopy); platelet factor 3; total platelet adenine nucleotide; and clot retraction of PRP.

Platelet aggregation studies were normal with arachidonic acid and ristocetin. The response to 6 μmol/L ADP and 1 to 2 μg/mL collagen was normal in most patients. Occasional reduced aggregation with ADP and collagen was noted in several family members, but this abnormality was not consistently found; on some occasions, the abnormality was observed when the PRP platelet count was less than 250 × 10^9/L. However, all family members with bleeding symptoms previously tested for aggregation defects (n = 12; IV 5, IV 9, IV 19, IV 20, IV 21, IV 23, V 5, V 15, V 22, V 23, V 33, and VI 10) had absent aggregation in response to epinephrine (6 to 10 μmol/L).

**Laboratory Investigations**

**Platelet multimerin analyses.** Quantitative analyses of platelet multimerin, using an ELISA, indicated that patients with factor V Quebec (n = 11 tested) were deficient in multimerin, with levels that ranged from 5% to 27% (mean, 12.5%) of the normal pool (normal range, mean ± 3 SD, calculated using log-transformed data, 45% to 214%). In contrast, the multimerin levels were normal in the asymptomatic family members (n = 4 tested) and in the unrelated patient with factor V deficiency (Fig 3). The multimerin levels were normal in the 18 unrelated patients undergoing investigations for bleeding disorders, including the patients with thrombocytopenia.

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**Fig 2.** Bleeding complications in an individual with factor V Quebec. (A) Bleeding, requiring hospitalization and transfusion support, with eruption of a wisdom tooth. (B) Hematoma after a ride on a snowmobile. (C) X-ray of both ankles, taken at 30 years of age, indicating arthropathy in the left ankle joint secondary to recurrent hemarthroses.
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Patient’s multimerin analyzed using either monoclonal or polyclonal antimultimerin, even when prolonged (overnight) exposures were used for analyses. The patient’s multimers (analyzed using nonreduced multimer gels and either monoclonal or polyclonal antisera) were normal in mobility and in distribution, indicating a quantitative deficiency (Fig 4).

Investigations of platelet factor V: The patient with platelet and plasma factor V deficiency had low platelet factor V antigen levels in all ELISA; however, variable results were obtained in the factor V Quebec patients, with some assays failing to discriminate between symptomatic and asymptomatic family members (Table 1 and Fig 5). The ELISA, using two different polyclonal antihuman factor V antisera (Fig 5), indicated significant reductions in platelet factor V antigen in the symptomatic family members (levels, 12% to 18% of the normal pool; normal controls, mean ± 2 SD, 48% to 148%).

Platelet factor V is known to be heterogenous in its molecular weight. Immunoblot analyses of platelet lysates, using SDS-PAGE and polyclonal antisera, indicated a normal pattern of variably sized platelet factor V molecules in the lysates from controls and asymptomatic family members. However, reduced quantities of platelet factor V were detected in the lysates from the patient with factor V deficiency and from the factor V Quebec patients (Fig 6). Comparisons

Immunoblot analyses confirmed the deficiency of platelet multimerin in the factor V Quebec patients (Fig 4). Evaluation of their multimerin subunits using either monoclonal or polyclonal antimultimerin and reduced SDS-PAGE (Fig 4) indicated that their multimerin subunits were of normal size. No evidence of proteolytic degradation was detected in the

![Immunoblot analyses of platelet multimerin. Samples of whole platelet lysate were separated using reduced SDS-PAGE (top panel) or nonreduced agarose-acrylamide gels (middle and lower panels) and immunoblotted using monoclonal (middle and lower panels) or polyclonal antimultimerin (top panel) and chemiluminescence for protein detection. Symptomatic individuals in the factor V Quebec pedigree are indicated by an asterisk. Samples of the normal pool (NP; diluted to 10% and 40%) are shown for comparison. The multimer pattern in the patient samples resembled shorter exposures of the normal pool samples (lane NP, lower panel).](image-url)

![Platelet factor V antigen levels. Results obtained using an ELISA (capture antibody, horse antihuman factor V; detection, sheep antihuman factor V) for controls, individuals from the factor V Quebec family, and an unrelated factor V deficient patient (Factor V Deficiency). The bars indicate the normal range (mean ± 2 SD).](image-url)
of the samples from the factor V Quebec patients to diluted samples of the normal pool or controls indicated differences in the relative prevalence of the different factor V bands in the factor V Quebec patients (Fig 6, middle and right panels). In addition, a doublet at 133 kD was seen in the control samples but not in the patients’ samples, even on prolonged exposures. These data indicate both qualitative and quantitative platelet factor V abnormalities.

Assessments of other platelet proteins. No deficiencies were detected in the factor V Quebec patients’ platelet stores of von Willebrand factor (n = 3 tested; range, 74% to 90% of the normal pool; mean ± 2 SD for 20 controls, 40% to 148%). The β-thromboglobulin antigen levels in the factor V Quebec patients (n = 3 tested; range, 71% to 83% of the normal pool) were similar to the asymptomatic family members and to the unrelated controls (n = 6 tested; range, 63% to 83% of the normal pool). No deficiencies were detected in their platelet thrombospondin antigen levels (n = 3 tested; range, 92% to 252% of the normal pool; mean ± 2 SD for 14 controls, 48% to 188%). The levels of platelet fibrinogen antigen in the patients (n = 3 tested) ranged from 60% to 87% of the normal pool (mean ± 2 SD for 22 controls, 54% to 158%). These data excluded gray platelet syndrome, a disorder characterized by thrombocytopenia and a generalized, α-granular protein deficiency, as the cause of their bleeding disorder.

Despite the normal levels of von Willebrand factor, β-thromboglobulin, thrombospondin, and fibrinogen, the non-reduced/reduced analysis of biotinylated proteins in platelet lysate and releasate from the factor V Quebec patients indicated that there were abnormalities in other platelet proteins (Fig 7). The immunoprecipitates of their membrane GPIb-IX and GPVIβ1 were normal, indicating that not all platelet glycoproteins were abnormal (Fig 7). The abnormalities in the mobility of high molecular weight (>300 kD) nonreduced platelet proteins (Fig 7) led us to evaluate their thrombospondin, von Willebrand factor, and fibrinogen (Fig 8). Immunoblot and immunoprecipitation studies indicated qualitative abnormalities in factor V Quebec patients’ platelet thrombospondin, with both intact and proteolyzed forms evident (Fig 8). The abnormal thrombospondin was observed in the samples of labeled platelet releasate, labeled lysate, washed platelet lysates, and solubilized PRP pellets (prepared from samples collected in anticoagulant with protease and activation inhibitors and immediately pelleted and lysed into buffer containing protease inhibitors). Thrombospondin degradation was evident in the lysates from all symptomatic (n = 11), but not asymptomatic, family members tested. The

### Table 1. Comparison of Platelet Factor V Antigen Levels Measured by Different Assays

<table>
<thead>
<tr>
<th>Individual</th>
<th>V 22*</th>
<th>V 23*</th>
<th>VI 10*</th>
<th>VI 9</th>
<th>VI 8</th>
<th>FV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA (monoclonal antisera)†</td>
<td>3.2</td>
<td>QNS</td>
<td>1.1</td>
<td>1.2</td>
<td>2.3</td>
<td>0</td>
</tr>
<tr>
<td>ELISA (polyclonal antisera)†</td>
<td>0.2</td>
<td>QNS</td>
<td>0.6</td>
<td>0.9</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td>RIA‡</td>
<td>1.7</td>
<td>0.7</td>
<td>2.0</td>
<td>1.6</td>
<td>1.5</td>
<td>NT</td>
</tr>
</tbody>
</table>

Factor V antigen levels (in micrograms per 10⁹ platelets) in symptomatic (noted with an asterisk) and asymptomatic factor V Quebec family members and in an unrelated patient with factor V deficiency (FV*) are shown. Quantitations were performed on aliquots of the same samples, using purified plasma factor V as the standard.

Abbreviations: NT, not tested; QNS, quantity insufficient for analysis in all assays.
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presence of degraded thrombospondin in patients’ platelet releasate indicated that the abnormal proteolysis was not caused by the detergent solubilization. Comparisons of the factor V Quebec patients to other individuals with disorders of platelet production and consumption indicated a distinct pattern of thrombospondin proteolysis in the platelet lysates from the factor V Quebec patients (Fig 8).

Similar proteolytic abnormalities were identified in the platelet von Willebrand factor of the factor V Quebec patients, with a relative increase in a 175-kD proteolytic fragment (Fig 8) in samples of washed platelet lysates or solubilized PRP pellets from all 11 symptomatic family members studied. This pattern of proteolysis was not seen in the lysates from the asymptomatic family members or in the lysates from nine other individuals with thrombocytopenia due to a variety of immune and nonimmune causes (Fig 8).

In addition to the abnormalities in megakaryocyte-synthesized α-granular proteins, the platelets from the factor V Quebec patients contained abnormal fibrinogen, with proteolytic fragments evident in both immunoblot (Fig 8) and immunoprecipitation (Fig 7) analyses. The platelet fibrinogen proteolytic degradation in the factor V Quebec patients was also evident in samples of lysate prepared from platelets pelleted from PRP; these samples also contained undegraded, plasma fibrinogen (Fig 8). Degraded fibrinogen was found in the platelet lysate lysates from all 11 symptomatic family members evaluated. The lysates prepared from controls, asymptomatic family members, and unrelated individuals with thrombocytopenia did not show evidence of fibrinogen degradation (Fig 8).

Studies of platelet α-adrenergic receptor numbers and function. The factor V Quebec patients previously tested for aggregation defects (n = 12; IV 5, IV 9, IV 19, IV 20, IV 21, IV 23, V 5, V 15, V 22, V 23, V 33, and VI 10) had absent aggregation in response to 6 to 10 μmol/L epinephrine. The epinephrine aggregation response of asymptomatic family members (n = 3 tested; IV 1, IV 4-1, and V 14) were normal. Additional epinephrine aggregation testing was performed on samples from patients IV 17, IV 21, IV 23, V 22, V 23, and V 33 and from IV 4-1, an unaffected family member. The absent epinephrine aggregation (6 μmol/L) was confirmed in all of the patients tested (Fig 9). The unaffected family member IV 4-1 and unrelated controls had a normal epinephrine aggregation response. When testing was performed at higher doses of epinephrine (40 μmol/L final), variable responses were noted, with the patients exhibiting either absent aggregation (IV 17, IV 21, IV 23, and V 22 [one occasion] and V 33) or an extremely prolonged lag phase (4 to 9 minutes; no primary wave evident) before full platelet aggregation occurred (82% to 97%; V 22 [one occasion] and V 23 [both occasions tested]; Fig 9). The abnormal epinephrine aggregation response suggested that there might be additional abnormalities affecting the factor V Quebec patients’ platelet α2-adrenergic receptors. This possibility was investigated using [3H] yohimbine binding studies and Scatchard analyses to quantitate the receptor numbers and binding affinities. The factor V Quebec patients’ (n = 4 tested; IV 17, IV 21, IV 23, and V 33) α2-adrenergic receptors ranged from 220 to 296 receptors/platelet; kd, 2.3 to 4.8 nmol/L (Fig 9). Similar values were obtained in studies of the asymptomatic family member (IV 4-1; receptors, 270/platelet; kd, 2.9 nmol/L; Fig 9) and unrelated controls (n = 6 tested; receptors, 211 to 426/platelet; kd, 2.3 to 4.8 nmol/L). The receptor numbers and binding affinities for both patients and controls were similar to normal values reported by other investigators.

DISCUSSION

About 5 years ago, we identified a novel platelet and endothelial cell protein that was designated as multimerin, based on its massive size (millions of daltons) and repeating, disulfide-linked homomultimeric structure. Our subsequent studies indicated that multimerin is synthesized by megakaryocytes, stored inside α-granules, secreted on platelet activation, and binds to the surface of activated platelets. In more recent studies, we identified that multimerin is stored, within platelets, as a complex with factor V; how-

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ever, studies of activated platelets indicated that these complexes dissociate after platelet activation by thrombin. These data led us to consider that the multimerin in platelet α-granules might function as a carrier protein for factor V. In addition, the primary structure of multimerin, obtained by cloning and sequencing of the multimerin cDNA, suggested possible adhesive functions for multimerin. We postulated that knowledge of a human deficiency state might provide insights into the role of this protein in vivo.

Several years ago, Tracy et al described an autosomal dominant bleeding disorder that they termed factor V Quebec. Although these patients were found to have adequate levels of plasma factor V for hemostasis, abnormalities were observed in their platelet factor V function, which was corrected by exogenous factor Va. Our observation of the association between platelet factor V and multimerin in normal platelets led us to investigate these individuals and an unrelated patient with plasma and platelet factor V deficiency for multimerin abnormalities.

We found that the factor V Quebec patients were deficient in their platelet stores of multimerin, with levels ranging from 5% to 27% of the normal pool, which were all significantly below our normal range of 45% to 214% (Fig 3). Immunoblot analyses of subunit and multimer gels confirmed that their platelets contained reduced quantities of multimerin (Fig 4), and no proteolytic degradation was detected. Normal multimerin was observed in the asymptomatic family members and also in the patient with platelet and plasma factor V deficiency, indicating that platelet factor V is not required to store multimerin. The multimer pattern of samples from the deficient patients was normal, indicating a quantitative deficiency of multimerin.

Additional investigations were performed to evaluate the nature of the platelet factor V defect in the individuals with factor V Quebec. Compared with plasma factor V, platelet factor V is known to be heterogeneous due to variable proteolysis in the activation peptide region. Platelet factor V normally consists of peptides, ranging in size between 150 and 330 kD, that are capable of generating factor Va. Immunoblot analyses using polyclonal antibodies against human factor V indicated a marked reduction in all forms of platelet factor V antigen between 150 and 330 kD in the individuals with factor V Quebec and in the unrelated individual with factor V deficiency (Fig 6). Many patients with inherited factor V deficiency have reduced factor V antigen and activity; however, individuals with factor V antigen levels that are greater than their factor V activity levels have been reported. We observed variable quantities of platelet factor V antigen in each of the factor V Quebec individuals when measured by different assays, suggesting that their platelets contain factor V degradation products.

Gray platelet syndrome is an inherited bleeding disorder characterized by thrombocytopenia and a deficiency of soluble α-granule proteins, including von Willebrand factor, β-thromboglobulin, fibrinogen, and albumin. Patients with this disorder have abnormal platelets that appear gray in stained blood films and exhibit abnormal, empty α-granules on electron microscopy. Similar to patients with gray platelet syndrome, a number of the factor V Quebec individuals were also thrombocytopenic; however, their platelets contained...
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normal quantities of von Willebrand factor, β-thromboglobulin, thrombospordin, and fibrinogen antigen, and light and electron microscopy indicated that their platelet morphology was normal.

Because the data suggested that the platelet factor V in the factor V Quebec patients was degraded, we searched for evidence of proteolysis in other platelet proteins. In contrast to the deficiency of specific α-granular components in nonreduced/reduced analyses of gray platelet syndrome platelets, a markedly abnormal nonreduced/reduced profile of platelet proteins was found in the labeled lysate and releasate from the factor V Quebec patients (Fig 7). Immunoblot and immunoprecipitation analyses indicated that their platelets contained intact and proteolyzed forms of von Willebrand factor and degraded thrombospordin (Fig 8), despite normal antigen levels. In addition to the abnormalities detected in megakaryocyte-synthesized α-granular proteins, their platelets also contained proteolyzed fibrinogen (Figs 7 and 8), a plasma protein that is also stored in α-granules. These data indicate a defect that affects both megakaryocyte-synthesized and plasma-derived α-granular proteins. Because the degraded proteins were also found in their platelet releasate, these findings indicate that the proteolyzed α-granular proteins are contained within a releasable storage pool, likely within α-granules.

The abnormal platelet thrombospordin, von Willebrand factor, and fibrinogen accounted for some of the glycoprotein abnormalities identified in nonreduced/reduced migration studies of the patients’ platelet proteins. Some but not all of the abnormal bands present in the patients’ samples were identified, suggesting that other platelet proteins may be altered in this disorder. Although immunoprecipitates of their platelet membrane GPIb-IX and GPαIbβ3 were normal, further studies are required to determine if the glycoprotein abnormalities are restricted to α-granular constituents. We considered that some of these changes could be secondary to abnormalities in platelet production or consumption. However, the fibrinogen, von Willebrand factor, and thrombospordin proteolysis were not detected in the samples from unrelated individuals with a variety of platelet production and consumption disorders, which included inherited thrombocytopenia, immune thrombocytopenia, amegakaryocytic thrombocytopenia, aplastic anemia, and myelodysplastic syndrome.

Studies of platelet function have shown a consistent, abnormal aggregation response of the factor V Quebec platelets (n = 12 evaluated). Their platelets failed to aggregate in response to 6 to 10 μmol/L epinephrine, despite normal aggregation responses to other agonists. The three asymptomatic family members tested had a normal aggregation response to epinephrine. In addition, the patients had either absent aggregation or an extremely prolonged lag phase (without primary aggregation) in response to high epinephrine doses.

Epinephrine is known to activate platelets by binding to α2-adrenergic receptors, leading to activation of GPαIbβ3,
fibrinogen binding, and platelet aggregation.\textsuperscript{58,59} Although the physiologic significance of epinephrine-induced platelet activation in vivo is uncertain, isolated, abnormal epinephrine aggregation has been observed in patients with myeloproliferative disorders, healthy individuals, and, rarely, in other disorders.\textsuperscript{45,46,60,61} Autosomal dominant, familial deficiencies of platelet $\alpha_2$-adrenergic receptor numbers associated with a reduced aggregation response to epinephrine have also been reported; however, this abnormality was not associated with bleeding.\textsuperscript{45,60,61} Recently, a high prevalence (16\%) of abnormal epinephrine aggregation and reduced quantities of platelet $\alpha_2$-adrenergic receptors was reported in Japanese individuals.\textsuperscript{62} These individuals had reduced (approximately 5\% to 30\%), but not absent, aggregation in response to high epinephrine doses.\textsuperscript{62} In contrast to the low platelet $\alpha_2$-adrenergic receptors found in these other conditions associated with abnormal epinephrine aggregation, the factor V Quebec patients had normal numbers of platelet $\alpha_2$-adrenergic receptors and the binding affinity of their platelet receptors for the $\alpha_2$-adrenergic receptor antagonist yohimbine was normal. Because the epinephrine aggregation defect was found in all of the factor V Quebec patients tested ($n = 12$), we postulate that the bleeding disorder and defective epinephrine aggregation are the result of a single genetic mutation. Investigations are in progress to determine if their abnormal epinephrine aggregation response is due to a defect in activation of the fibrinogen receptor or impaired platelet-platelet interaction. The contribution of their abnormal $\alpha$-granular proteins to this defect is uncertain as patients with gray platelet syndrome have had a either normal or slightly delayed aggregation response to standard concentrations of epinephrine.\textsuperscript{51,52} Although epinephrine aggregation studies have been used to help identify the affected members of this family, the reported high prevalence of abnormal epinephrine aggregation in healthy individuals may limit the usefulness of this testing.

These studies indicate that individuals with factor V Quebec have abnormal platelet multimerin, factor V, thrombospondin, von Willebrand factor, and fibrinogen, but the molecular defect that leads to the multiple abnormalities has not yet been identified. The abnormalities in some platelet proteins (factor V, von Willebrand factor, thrombospondin, and fibrinogen) indicate that proteolysis of $\alpha$-granular proteins is part of the pathogenesis and suggest that the disorder may be due to an abnormal protease or a deficient protease inhibitor. However, only quantitative abnormalities were found in their multimerin. It is possible that significant quantities of degraded multimerin, lacking the epitopes for the monoclonal and polyclonal multimerin antisera, could be present in the patients’ platelets. However, the presence of high molecular weight multimerin multimers, despite antigen levels as low as 5\%, suggests that factors other than proteolytic degradation are the cause of their multimerin deficiency. Possible explanations include an effect of their genetic disorder on multimerin expression or an indirect effect of the proteolytic process on the ability to synthesize and store multimerin. In view of the multiple protein abnormalities identified, the contribution of the individual protein abnormalities to their bleeding and the relationship between the multimerin deficiency and abnormalities in platelet factor V are uncertain.

In addition to the glycoprotein abnormalities and aggregation defects identified, the factor V Quebec patients were noted to have a significantly lower platelet count than controls, although overlap with the normal range was noted. In all patients evaluated, the mean platelet volume was normal. No relationship has been found between their platelet count and severity of bleeding symptoms, and serious bleeding episodes have occurred in individuals who were not thrombocytopenic. One of the factor V Quebec patients had platelet survival studies performed previously; however, no abnormalities were identified. The abnormal platelet counts suggest that their genetic defect may also influence platelet production and/or destruction, but the explanation for their low platelet counts is, at present, uncertain.

These studies identify a new form of autosomal-dominant, qualitative platelet disorder characterized by mild thrombocytopenia to low normal platelet counts; a quantitative deficiency of platelet multimerin; quantitative and qualitative abnormalities of platelet factor V; abnormal proteolysis of platelet thrombospondin, fibrinogen, and von Willebrand factor; and an associated epinephrine aggregation defect. Diseases associated with abnormalities in many platelet glycoproteins are rare; this disorder and gray platelet syndrome are the only known examples.

An important issue remains unresolved, i.e., why do these patients bleed? The multiple platelet glycoprotein abnormalities suggest that their hemostatic defect may be due to many factors. However, their failure to respond to platelet transfusions, even when fresh platelets or massive quantities are transfused, suggests that other factors, separate from replacing deficient or abnormal platelet proteins, are important determinants of bleeding in this disorder. Quantitative analyses of platelet multimerin may be useful in distinguishing this disorder from other qualitative platelet disorders.

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An autosomal dominant, qualitative platelet disorder associated with multimerin deficiency, abnormalities in platelet factor V, thrombospondin, von Willebrand factor, and fibrinogen and an epinephrine aggregation defect

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