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Type 2B von Willebrand Disease Associated With the Release of Platelet Agglutinates From Megakaryocytes in the Bone Marrow

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Summary

We report a child with thrombocytopenia since birth, circulating platelet agglutinates, and a tendency to bleed. A bone marrow aspirate revealed large platelet clumps within the bone marrow and megakaryocyte nuclei surrounded by halos of clumped platelets. Laboratory evaluation revealed type 2B von Willebrand disease. Gene sequencing revealed a G to C mutation at base 3923 of the VWF gene. This mutation was previously described in a family with circulating platelet clumps and abnormal megakaryopoiesis with release of clumped platelets in culture. This same mutation was previously described in a family with circulating platelet aggregates and abnormalities of platelet release from megakaryocytes in vitro. Presence of megakaryocytes with halos of clumped platelets in our patient suggests that platelet agglutinate occurs in the bone marrow in some type 2B von Willebrand disease patients.

Keywords

von Willebrand disease; type 2B; megakaryocyte; platelet clumps; pseudothrombocytopenia

Congenital thrombocytopenia is rare, and the underlying cause can be difficult to uncover.¹ Platelets are produced as the result of a series of steps.^{2,3} Multipotent progenitors differentiate into committed blast-forming unit or colony-forming unit—megakaryocytes, proliferative cells that have committed to the megakaryocyte lineage. Individual megakaryocytes stop dividing, but the nucleus continues to replicate DNA, a process called endoreduplication. The result is a large cell with a polyploid nucleus. Finally, demarcation membranes develop separate individual platelets. Platelets are released in the form of proplatelet processes, which unwind from the plateletlike beaded string from a ball of string. Individual platelets (the beads) separate from each other in the circulation. Problems can arise at any of these stages of platelet development. Recently, the presence of specific

genetic mutations have been directly linked to morphologic abnormalities of megakaryocytes.⁴⁻⁶

The presence of clumped platelets on a peripheral smear can result from in vitro platelet aggregation that occurs after the blood is drawn from the patient. Pseudothrombocytopenia occurs when antibodies in the patients' plasma are activated when the blood is placed into a tube containing ethylene diamine tetraacetic acid (EDTA), leading to platelet aggregation.⁷⁻¹⁰ This aggregation leads to low platelet concentration when measured on an automated cell counter, and the presence of clumped platelets on a peripheral blood smear. The diagnosis of pseudothrombocytopenia is made by comparing blood smears collected in EDTA with smears collected in heparin or without anticoagulant. Patients with pseudothrombocytopenia do not have bleeding problems. Clumped platelets in association with increased bleeding can be associated with pathologic states where platelets clump in the circulation and cause thrombosis or bleeding.

Type 2B von Willebrand disease (VWD) is one pathologic state that has been associated with clumped platelets in the circulation. VWD results from quantitative or qualitative deficiencies in von Willebrand factor. Von Willebrand factor carries the clotting protein factor VIII, and also serves to tether platelets to sites of vascular injury. Patients with type 2B VWD have mutations in von Willebrand factor that lead to increased affinity to the platelets leading to agglutination and removal from circulation.¹¹ The clearance of these agglutinates results in thrombocytopenia and loss of the high molecular weight multimers of VWF from the circulation. This hyperactive von Willebrand factor is the result of gain-of-function mutations that cluster within exon 28 of the VWF gene. Type 2B VWD should be suspected in patients with low-high molecular weight multimer levels associated with thrombocytopenia, or VWD patients who develop thrombocytopenia after treatment with arginine vasopressin. Type 2B VWD should be differentiated from "pseudo" or "platelet-type" VWD, which is caused by gain of function mutations of the VWF receptor on the platelets themselves, and differentiating these 2 disorders has been facilitated by genetic sequencing of the VWF and GP1b genes.^{12,13} Circulating platelet agglutinates have only been reported in a subset of type 2B VWD patients.^{14,15} In addition to the functional abnormalities in von Willebrand factor, these mutations can lead to other morphologic abnormalities of platelets, such as giant platelets.^{16,17} Here, we describe a child with type 2B VWD with morphologic abnormalities of both his platelets and megakaryocytes.

RESULTS/DISCUSSION

We referred a small child for evaluation of thrombocytopenia and a tendency to bruise and bleed from his oral mucous membranes. His case had previously been reported as an example of pseudothrombocytopenia that was independent of EDTA.¹⁸ He was born at 26 weeks by caesarean section for suspected abruption and preterm labor. His perinatal course was complicated by mild broncho-pulmonary dysplasia and retinopathy of prematurity. His examination was remarkable for the presence of numerous purpura and petechiae on his face, limbs and trunk, and for the lack of hepatosplenomegaly and orthopedic abnormalities. His peripheral platelet counts ranged from 5000 to 30,000/mL. When his thrombocytopenia persisted beyond the perinatal period, he had 2 bone marrow aspirates performed before

referral to our center. He had numerous platelet agglutinates in the peripheral blood smear (Figs. 1A, B). His bone marrow aspirate showed numerous large platelet clumps and megakaryocyte nuclei surrounded by clumped platelets (Figs. 1C, D).

Because of the patient's bleeding tendency, the presence of clumped platelets in the peripheral circulation and lack of schistocytes, we considered the diagnosis of type 2B VWD. Circulating platelet agglutinates have been described in at least 2 families in association with type 2B VWD.^{14,15} Although the VWF antigen level, measured using an immunoturbidimetric method, was in the reference range [54 IU/dL, normal range (NR) 45 to 170 IU/dL], the activity (as assessed by the ristocetin cofactor assay) was reduced (24 IU/dL, NR >50 IU/dL). Factor VIII activity was low-normal (63 IU/dL NR 60 to 200 IU/dL), and was consistent with the VWF antigen concentration. Because the ratio of VWF activity to VWF antigen was <0.7, the result was suggestive of type 2 VWD,¹⁹ and multimer studies were performed. The multimer pattern was abnormal with the absence of high molecular weight multimers. This result is consistent with either type 2B VWD, some types of type 2A VWD, or platelet type VWD. VWF studies were repeated at the Blood Center of Wisconsin, Milwaukee, WI. Factor VIII activity was 27 IU/dL (NR 60 to 195), ristocetin cofactor activity was <10 IU/dL (NR 54 to 279), and VWF antigen level was 26 (NR 50 to 240). Although the second set of studies was quantitatively lower than the first, the relative proportions of VWF ristocetin cofactor and factor VIII level to the VWF antigen are similar to the initial studies. It is well known that acute phase reaction, stress, and hormonal effects may lead to quantitative variation of VWF levels. Electrophoresis confirmed the absence of high and intermediate weight multimers (Fig. 2). Increased binding of patient VWF to formalin-fixed normal platelets was observed at a low concentration of ristocetin, consistent with type 2B VWD.²⁰ Gene sequencing of VWF exon 28 revealed a heterozygous G to C mutation at base 3923 encoding replacement of arginine 1308 with proline (R545P in terms of older nomenclature).

Nurden et al¹⁴ recently reported a family with the same mutation who had a variant of type 2B VWD associated with a bleeding tendency, profound thrombocytopenia, and circulating platelet agglutinates. Cultured CD34 expressing progenitors from the peripheral blood produced abnormal megakaryocytes that showed incomplete platelet release resulting in platelets that never separated from their proplatelet processes. They proposed that the platelets were released from the immature megakaryocytes in an apoptotic phase of proplatelet formation, and that these platelets circulated as agglutinates. Further, they suggested that the presence of these platelet clumps explained the very low circulating platelet levels and bleeding tendency. However, they did not examine bone marrow from members of this family. Our study confirms the association and demonstrates that at least 1 patient with this particular mutation has abnormal thrombopoiesis with evidence of platelet clumps surrounding megakaryocyte nuclei in vivo.

VWF is produced in megakaryocytes and endothelium. Nurden proposes 2 potential mechanisms that might lead to the release of clumped platelets from megakaryocytes; first, that von Willebrand factor is released from megakaryocytes but binds proplatelets as they extend from the megakaryocyte surface, or second, that the abnormal VWF protein spontaneously binds to VWF receptor (GP1ba) during its passage through the Golgi in

platelets resulting in the 2 proteins being carried together to the cell surface.¹⁴ In the first case, clumping within the marrow could be related to plasma VWF that has been carried to the bone marrow, and/or VWF released from the marrow sinusoidal endothelium where megakaryocytes reside, as there is abluminal and luminal subendothelial release of VWF.²¹

Our case report extends and validates Nurden's findings¹⁴ by demonstrating that megakaryopoiesis in the bone marrow (rather than in CD34⁺ cell culture) is abnormal in at least 1 patient with type 2B VWD who has the R1308P mutation, and suggests that this mutation confers a predictable phenotype. Before we knew the genotype of this patient, we were aware of the similarities between this patient's megakaryocytes and those of the family described in Nurden's study. We were intrigued to find that our patient and the family described in Nurden's study shared a VW factor mutation associated with the presence of clumped platelets around megakaryocytes. Recognizing that isolated platelet agglutinates and halos of platelets clumped around megakaryocyte nuclei can be a sign of type 2B VWD will be helpful in making the correct diagnosis in patients with congenital thrombocytopenia.

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FIGURE 1.

Wright-stained peripheral smear and bone marrow aspirate of patient with type 2B von Willebrand disease. A and B, Peripheral smear demonstrates platelet clumps, which led to consideration of the patient's diagnosis. C and D, Platelet clumps surround megakaryocytes and are present in the marrow aspirate.

FIGURE 2.

Agarose gel showing von Willebrand multimers: normal plasma, our patient (with 3923G>A) producing the amino acid substitution 1308 arginine>proline and a patient with known type 2B von Willebrand disease and a 3946 G>A, which encodes for the substitution 1316 valine>methionine. Controls were run on the same gel, with additional patients between our patient and the type 2B control, which have been removed using photoediting and replaced by a vertical line.