Platelet-Type von Willebrand Disease: A Rare, Often Misdiagnosed and Underdiagnosed Bleeding Disorder

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ABSTRACT

Platelet-type von Willebrand disease (PT-VWD) is an autosomal dominant rare bleeding disorder characterized by hyperresponsive platelets. This inherent platelet function defect is due to a gain-of-function mutation within the *GP1BA* gene coding for the platelet surface glycoprotein Ib alpha protein, the receptor for the adhesive protein von Willebrand factor (VWF). The defect results in excessive and unnecessary platelet–VWF interaction with subsequent removal of the hemostatically efficient high molecular weight VWF as well as platelets from the circulation, leading to thrombocytopenia and bleeding diathesis. Patients with PT-VWD present with mild to moderate mucocutaneous bleeding, which becomes more pronounced during pregnancy and following aspirin ingestion or drugs that have antiplatelet activity. Laboratory testing shows low VWF:ristocetin cofactor and low or normal VWF:antigen and characteristically an enhanced ristocetin-induced platelet agglutination (RIPA). These laboratory features are also indicators of the closely similar and more common bleeding disorder type 2B VWD. Simplified RIPA mixing assays, cryoprecipitate challenge, and flow cytometry can differentiate between the two disorders. However, the gold standard is to identify mutations within the *VWF* gene (indicating type 2B VWD) or the platelet *GP1BA* gene *(*confirming PT-VWD). Treatment is based on making a correct diagnosis of PT-VWD where platelet concentrates instead of VWF/factor VIII preparations should be administered. A recent fairly large retrospective/prospective registry-based international study showed that PT-VWD is very rare, likely to be misdiagnosed as type 2B VWD or idiopathic thrombocytopenic purpura, and represents 15% of type 2B VWD diagnoses.

KEYWORDS: Glycoprotein 1bα, type 2B VWD, registry, RIPA, PT-VWD mouse, platelet concentrate

Originally described in 1982, by Weiss[1] and coworkers and initially named pseudo von Willebrand disease (pseudo VWD), this mild mucocutaneous bleeding disorder, currently known as platelet-type von Willebrand disease (PT-VWD), is still a challenging diagnosis to make despite the almost 3 decades that have passed. Although several reports have recently focused on the diagnostic dilemma and the significance of

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appropriate differentiation from the closely similar disorder type 2B VWD (reviewed in Othman, 2007),[2] PT-VWD is still likely an underdiagnosed/misdiagnosed bleeding disorder. A correct diagnosis and treatment are important because the disease represents an impaired interaction between platelets and the adhesive protein von Willebrand factor (VWF), a critical step in primary hemostasis. Relatively few reports are available with respect to the molecular pathology of this disease including the impact of some mutations as well as information about megakaryopoiesis. This indicates that further research studies are required to improve the understanding of the disease mechanisms and permit better diagnosis and treatment.

The first report of this bleeding disorder described four members from four generations of a family with a mild bleeding disorder.[1] The patients had intermittent thrombocytopenia, decreased plasma levels factor VIII/VWF, absence of high molecular weight (HMW) forms of VWF in the plasma (but normal multimeric structure in the platelets), and increased ristocetin-induced platelet agglutination (RIPA), as in type 2B VWD. However, unlike the abnormality in type 2B disease, which is a defect of VWF, the basic defect in this family was in their platelets, which absorbed HMW VWF multimers at lower concentrations of ristocetin than did normal platelets. In addition, either in platelet-rich plasma or suspended in buffer, their platelets were aggregated by unmodified normal human plasma without ristocetin.

In the same year, Miller and Castella[3] described five patients representing three generations of a single family. Prolonged bleeding times, low to normal platelet counts, normal factor VIII coagulant (FVIII:C) activity, selective decrease of the HMW VWF multimers, and increased RIPA at low ristocetin concentrations were the characteristic features of the disease. Since then, several families have been described with the same bleeding phenotype. Up to date, 44 cases (31 females and 13 males) of 18 families are known to have PT-VWD.[4]

In this review, the basic molecular genetics, pathology, characteristic phenotype, frequency, and management of PT-VWD are briefly described.

PATHOLOGY AND MOLECULAR GENETICS OF PLATELET-TYPE VON WILLEBRAND DISEASE

The intrinsic defect of PT-VWD lies in the platelet surface protein known as glycoprotein Ibα (GPIbα). This protein carries the binding site for the adhesive protein VWF and is part of a complex known as GPIb/IX/V on the platelet surface. The interaction between VWF and GPIbα does not normally take place, although both proteins are in contact in circulation. This interaction is stimulated by endothelial damage and the exposure of subendothelial matrices or in response to shear stress, which begins a series of events that lead to the formation of a thrombus to seal the site of injury. Following this, both VWF and platelets are cleared by macrophages in the reticuloendothelial system. Platelets of PT-VWD patients are inherently hyperresponsive and interact spontaneously with HMW multimers of VWF released from the endothelium. This leads to loss of these VWF multimers due to their deposition on the platelet surface together with thrombocytopenia caused by an increased removal of the bound platelets; reflecting two mechanisms behind the bleeding diathesis in PT-VWD.

Platelet *GP1BA* Gene

The human *GP1BA* gene, cloned in 1987 by Lopez and coworkers,[5] is a simple gene located on chromosome 17, spans a 2.4 kb of genomic DNA, and has two exons. The entire protein coding sequence is contained within exon 2 and the mRNA comprises ~1882 bp. The gene codes for a leader sequence of 16 aa (amino acids), a mature 610 aa protein, and a stop codon. There are also 42 nucleotides of 5′ noncoding sequence and 497 nucleotides of 3′ noncoding sequence, including the poly A tail.[5] The cloned mouse counterpart was reported by Ware and coworkers[6] and has a roughly similar size genomic DNA fragment (~2.8 kb), single exon encoding a 734-residue precursor polypeptide, and a 75% sequence similarity at the amino-terminal domain. Mouse and human primary sequences diverge through a short linear sequence of the amino-terminal domain critical for the binding of human VWF and in the extracytoplasmic macroglycopeptide domains, reducing the overall sequence similarity to 70%. This results in variability in aggregation responses between human and mouse platelets, a fact that needs to be considered when performing platelet research studies.

GP1BA Mutations and Mechanisms of Gain of Function

The platelet inherent defect is due to a gain-of-function mutation in the *GP1BA* gene coding for the platelet surface GPIbα. Although the clinical disease was first described in 1982,[1,3] the first mutation known to cause the disease was described 9 years later, 4 years following the cloning of this gene.[5] To date, only four mutations have been identified within the *GP1BA* gene. The first mutation, Gly 233 Val, was described in 1991 by Miller and coworkers[7] and again in 1993 by Russell and Roth.[8] The second was a Met 239 Val described in 1995 by Takahashi and coworkers[9] and again as a de novo mutation in 1997.[10] The two amino acid residues 233 and 239 are located within a β-hairpin loop in the crystal structure of GPIbα.[11] The Gly 233 Val and Met 239 Val mutations have been proposed to stabilize the loop

conformation leading to an increased affinity for VWF. Recombinant expression of these mutations in heterologous cells has confirmed that the mutations result in an in vitro higher affinity between receptor and ligand and are responsible for the disease phenotype.[12–14] The third mutation involved another change at the 233 residue; a Gly 233 Ser and was described in 2003[14] and again in 2007.[15] The fourth mutation is a 27bp deletion and was described by Othman and coworkers[16] in 2005. This is the first and only mutation described outside the VWF binding region. The mutation affects the mucin-rich macroglycopeptide region, which forms a rigid stalk that extends the ligand-binding domain. The mutation was thought to restrict the mobility of the extracellular domain, which, in turn, increases sensitivity to ristocetin-induced VWF and results in gain of function.[16]

The crystal structure of the N-terminal domain of GPIbα[17] and that of the VWF A1 domain/GPIbα complex[11,18] has been a cornerstone in providing the basic explanation of the mechanisms of receptor binding to VWF and has indeed facilitated the understanding of the effects of the PT-VWD mutations. The positively charged VWF A1 domain contacts GPIbα at the negatively charged leucine-rich repeats (LRRs). The anionic sulphated region of GPIbα may also be involved in this interaction based on surface charge representation, but this role has not been confirmed. Evidence also suggests that four specific residues at the C-terminal flank, amino acids 225, 226, 228, and 241, bind the VWF A1 domain directly. It was found that conformational changes in both the receptor and its ligand is the prerequisite for binding to remove the steric hindrance made by the highly flexible loop projecting from the concave face (R-loop) and allow the fitting of the positively charged VWF A1 domain into the negatively charged concavity made by the LRR. Common PT-VWD mutations Gly233Val and Met239Val mutations fall within the R-loop. They act by favoring a more open conformation of the receptor or by stabilizing the interaction between the two molecules.[11,17] The macroglycopeptide region forms a stalk that extends the ligand-binding domain. Although recombinant expression of the macroglycopeptide region deletion mutation (outside the VWF binding domain) has proved the disease phenotype,[16] the exact mechanism has not been fully understood.

Gene, Protein, and Disease Nomenclatures

It is worth noting that the mutation nomenclature may vary among publications. According to the Human Genome Organization (HUGO) and the Human Gene Nomenclature Committee, which stems from HUGO, http://www.genenames.org/, and the affiliated Human Genome Variation Society, http://www.hgvs.org/, there would be a slight difference in the naming of the mutations and polymorphism compared with those that have been used historically and may still be referred to by investigators in literature. For details about the nomenclature issues, refer to Othman et al.[19] Compared with the term *pseudo VWD*, PT-VWD is more descriptive of the pathophysiology and has already achieved universal utility. It needs to be emphasized, however, that the disorder does not belong to the VWD classification and should always be listed under platelet function defects.

PLATELET-TYPE VON WILLEBRAND DISEASE PHENOTYPE

PT-VWD is an autosomal dominant disease. Patients with PT-VWD present with mild to moderate mucocutaneous bleeding such as nosebleeds, bleeding after dental extraction, and postsurgery. Bleeding becomes more pronounced during pregnancy and following aspirin ingestion or drugs that have antiplatelet activity.[20]

Most patients do not seek advice unless they have serious bleeds. Some also do not comply with routine clinic visits, which leave them at a risk of bleeding if they are hemostatically challenged in situations such as pregnancy, dental procedures, or minor operations.

The characteristic laboratory feature that raises the suspicion of PT-VWD is the enhanced RIPA test. Classically, this would indicate a response to ≤ 0.5 mg/mL of ristocetin; however, recent publications have raised awareness that some cases of 2B VWD respond only to intermediate low levels of ristocetin ($\sim$0.7 mg/mL).[21] Accordingly, this should also be feasibly considered in the context of future PT-VWD investigations. Other laboratory features include loss from plasma of HMW multimers. The plasma VWF testing usually shows discordance in functional versus. nonfunctional test parameters (i.e., low VWF ristocetin cofactor activity and/or collagen binding capacity compared with antigen). FVIII levels are normal or variably reduced depending on VWF levels.[22] Platelet count can be normal. However, mild or moderate intermittent thrombocytopenia is usually aggravated by conditions that increase the endogenous release of VWF such as pregnancy, stress, and infection. Platelet macrocytosis, possibly due to shortened platelet survival, increased turnover, and prolonged bleeding time, can be seen. Platelet clumping is often observed in blood smears and is caused by the spontaneous binding of circulating VWF to platelets.

The more commonly encountered type 2B VWD shares most of the clinical and laboratory features of PT-VWD, including the unique enhanced RIPA. The discrimination between type 2B and PT-VWD can be very difficult and has been the subject of many reports in the past 5 years.[2,19,23–29] Methods of discrimination

include RIPA mixing assay,[25] cryoprecipitate challenge,[29] and flow cytometry.[30] For detailed differentiation between the two disorders, refer to previous reviews.[24,31] The gold standard test for a definitive diagnosis of PT-VWD is to identify the mutations within the platelet *GP1BA* gene. DNA sequencing of the whole coding sequence of the gene would identify the mutations responsible for the disease.

Platelet-Type von Willebrand Disease Animal Model

A mouse model of PT-VWD (G233V) was developed in 2008.[32] The transgenic mouse expresses the human GP Ibα in murine platelets with the normal Gly233 codon replaced with a Val233 codon (hTg^{G233V}), whereas the wild-type mouse expresses the normal human GP Ib alpha subunit (hTg^{WT}).[33] The G233V mice mimic the human PT-VWD with enhanced RIPA and prolonged bleeding time. Other phenotypic features include a dramatic increase in splenic megakaryocytes and splenomegaly as well as a high bone mass phenotype. This model is useful to study the disease further in terms of platelet activation, dysfunction, megakaryopoiesis and defects on several other body systems in PT-VWD.

FREQUENCY OF PLATELET-TYPE VON WILLEBRAND DISEASE WORLDWIDE

VWD is known to be the most common mild bleeding disorder, estimated to occur in ~1% of the normal population.[34] Type 2B VWD constitutes ~5 to 10% of all VWD types depending on included cases.[35] PT-VWD is often misdiagnosed as type 2B VWD because of the close similarity at clinical and laboratory levels, and it is therefore not difficult to conceive that PT-VWD would be underestimated. In a recent international prospective/retrospective study, it was estimated that PT-VWD constitutes 15% of the total number of patients diagnosed with type 2B VWD.[36] This is also supported by previous small case study series.

Platelet-Type von Willebrand Disease Database/ international Registry-Based Project

In 2007, an international project with the overall aim of tracking PT-VWD patients in Canada and internationally was initiated. The project was planned to address two major questions: Is PT-VWD rare or underdiagnosed? How many cases are being misdiagnosed as type 2B VWD cases? The project was completed in 2010, and detailed results are now available.[36] Over 3 years, 110 samples/data from eight countries were analyzed. Forty-eight cases were initially diagnosed as putative type 2B/PT-VWD that carried exon 28 mutations consistent with type 2B VWD, 17 carried *GP1BA* mutations consistent with a PT-VWD diagnosis, 3 represented other VWD types (2A and 2M), and 5 expressed three nonpreviously published exon 28 mutations. Excluding 10 unaffected family members and 1 acquired VWD, 26 cases did not have mutations in either gene. The percentage of type 2B VWD diagnosis was 44%; the percentage of misdiagnosis of PT-VWD as 2B VWD was 15%. It is worth noting that the ISTH-SSC working group on VWF originally approved the international online database and registry for PT-VWD, which facilitated the project as currently available and supported (www.pt-vwd.org). The Web site will be maintained to continue to track patients worldwide and to improve the understanding of this rare but significant bleeding disorder.

MANAGEMENT OF PLATELET-TYPE VON WILLEBRAND DISEASE

How should we manage a rare bleeding disorder such as PT-VWD? The treatment decision is based on making a correct diagnosis and identification of patients and the distinguishing it from the closely related type 2B VWD diagnosis. RIPA and platelet mixing studies are critically important, provided the facilities and experience in interpretation are available. A simplified RIPA mixing test has previously been described[25] and is useful in the simultaneous identification of both type 2B VWD and PT-VWD. When there is difficulty in performing this test, genetic analysis should be performed. DNA can be sent to experienced laboratories. Examination of the mutations within the A1 domain of the *VWF* gene and in mutation-negative cases the full platelet *GPIBA* gene will provide conclusive evidence by identification of causal mutation within one or the other gene.

Although 2B VWD patients are treated successfully with VWF/FVIII concentrate preparations such as Humate P, given intravenously, patients with PT-VWD require platelet transfusions to treat bleeding episodes because of their inherent platelet defect. It is important to note that the use of VWF/FVIII concentrate preparations may aggravate thrombocytopenia and worsen the bleeding condition in PT-VWD. Desmopressin is contraindicated because it stimulates the release of HMW VWF multimers from endothelium, which will then bind to more platelets.[20] Recombinant activated FVII has been suggested[31] as suitable for management, but no information is available in the literature. Antifibrinolytic agents such as aminocaproic acid can be also used before dental procedures or minor operations.

REFERENCES

1. Weiss HJ, Meyer D, Rabinowitz R, et al. Pseudo-von Willebrand's disease. An intrinsic platelet defect with

aggregation by unmodified human factor VIII/von Willebrand factor and enhanced adsorption of its high-molecular-weight multimers. N Engl J Med 1982;306(6): 326–333
2. Othman M. Platelet-type von Willebrand disease and type 2B von Willebrand disease: a story of nonidentical twins when two different genetic abnormalities evolve into similar phenotypes. Semin Thromb Hemost 2007;33(8): 780–786
3. Miller JL, Castella A. Platelet-type von Willebrand's disease: characterization of a new bleeding disorder. Blood 1982; 60(3):790–794
4. Registry PT-VWD. Available at:http//www.pt-vwd.org. Accessed February 9, 2011
5. Lopez JA, Chung DW, Fujikawa K, Hagen FS, Papayannopoulou T, Roth GJ. Cloning of the alpha chain of human platelet glycoprotein Ib: a transmembrane protein with homology to leucine-rich alpha 2-glycoprotein. Proc Natl Acad Sci U S A 1987;84(16):5615–5619
6. Ware J, Russell S, Ruggeri ZM. Cloning of the murine platelet glycoprotein Ibalpha gene highlighting species-specific platelet adhesion. Blood Cells Mol Dis 1997;23(2): 292–301
7. Miller JL, Cunningham D, Lyle VA, Finch CN. Mutation in the gene encoding the alpha chain of platelet glycoprotein Ib in platelet-type von Willebrand disease. Proc Natl Acad Sci U S A 1991;88(11):4761–4765
8. Russell SD, Roth GJ. Pseudo-von Willebrand disease: a mutation in the platelet glycoprotein Ib alpha gene associated with a hyperactive surface receptor. Blood 1993; 81(7):1787–1791
9. Takahashi H, Murata M, Moriki T, et al. Substitution of Val for Met at residue 239 of platelet glycoprotein Ib alpha in Japanese patients with platelet-type von Willebrand disease. Blood 1995;85(3):727–733
10. Kunishima S, Heaton DC, Naoe T, et al. De novo mutation of the platelet glycoprotein Ib alpha gene in a patient with pseudo-von Willebrand disease. Blood Coagul Fibrinolysis 1997;8(5):311–315
11. Huizinga EG, Tsuji S, Romijn RA, et al. Structures of glycoprotein Ibalpha and its complex with von Willebrand factor A1 domain. Science 2002;297(5584):1176–1179
12. Murata M, Russell SR, Ruggeri ZM, Ware J. Expression of the phenotypic abnormality of platelet-type von Willebrand disease in a recombinant glycoprotein Ib alpha fragment. J Clin Invest 1993;91(5):2133–2137
13. Moriki T, Murata M, Kitaguchi T, et al. Expression and functional characterization of an abnormal platelet membrane glycoprotein Ib alpha (Met239 —> Val) reported in patients with platelet-type von Willebrand disease. Blood 1997;90(2): 698–705
14. Matsubara Y, Murata M, Sugita K, Ikeda Y. Identification of a novel point mutation in platelet glycoprotein Ibalpha, Gly to Ser at residue 233, in a Japanese family with platelet-type von Willebrand disease. J Thromb Haemost 2003;1(10): 2198–2205
15. Nurden P, Lanza F, Bonnafous-Faurie C, Nurden A. A second report of platelet-type von Willebrand disease with a Gly233Ser mutation in the GPIBA gene. Thromb Haemost 2007;97(2):319–321
16. Othman M, Notley C, Lavender FL, et al. Identification and functional characterization of a novel 27-bp deletion in the macroglycopeptide-coding region of the GPIBA gene resulting in platelet-type von Willebrand disease. Blood 2005;105(11):4330–4336
17. Uff S, Clemetson JM, Harrison T, Clemetson KJ, Emsley J. Crystal structure of the platelet glycoprotein Ib(alpha) N-terminal domain reveals an unmasking mechanism for receptor activation. J Biol Chem 2002;277(38): 35657–35663
18. Dumas JJ, Kumar R, McDonagh T, et al. Crystal structure of the wild-type von Willebrand factor A1-glycoprotein Ibalpha complex reveals conformation differences with a complex bearing von Willebrand disease mutations. J Biol Chem 2004;279(22):23327–23334
19. Othman M. Differential identification of PT-VWD from type 2B VWD and GP1BA nomenclature issues. Br J Haematol 2008;142(2):312–314; author reply 314–315
20. Miller JL. Platelet-type von Willebrand disease. Thromb Haemost 1996;75(6):865–869
21. Federici AB, Mannucci PM, Castaman G, et al. Clinical and molecular predictors of thrombocytopenia and risk of bleeding in patients with von Willebrand disease type 2B: a cohort study of 67 patients. Blood 2009;113(3): 526–534
22. Nurden AT. Qualitative disorders of platelets and megakaryocytes. J Thromb Haemost 2005;3(8):1773–1782
23. Favaloro EJ. 2B or not 2B? Differential identification of type 2B, versus pseudo-von Willebrand disease Br J Haematol 2006;135(1):141–142; author reply 143
24. Favaloro EJ. Phenotypic identification of platelet-type von Willebrand disease and its discrimination from type 2B von Willebrand disease: a question of 2B or not 2B? A story of nonidentical twins? Or two sides of a multidenominational or multifaceted primary-hemostasis coin? Semin Thromb Hemost 2008;34(1):113–127
25. Favaloro EJ, Patterson D, Denholm A, et al. Differential identification of a rare form of platelet-type (pseudo-) von Willebrand disease (VWD) from type 2B VWD using a simplified ristocetin-induced-platelet-agglutination mixing assay and confirmed by genetic analysis. Br J Haematol 2007;139(4):623–626
26. Whalley IN, Perry DJ. 2B or not 2B? Differential identification of type 2B, versus pseudo von Willebrand disease. Br J Haematol 2007;136(2):345; author reply 345–346
27. Othman M, Lillicrap D. Distinguishing between non-identical twins: platelet type and type 2B von Willebrand disease. Br J Haematol 2007;138(5):665–666
28. Othman M, Hamilton A. Platelet-type von Willebrand disease: results of a worldwide survey from the Canadian PT-VWD project. Acta Haematol 2010;123(2):126–128
29. Enayat MS, Guilliatt AM, Lester W, Wilde JT, Williams MD, Hill FG. Distinguishing between type 2B and pseudo-von Willebrand disease and its clinical importance. Br J Haematol 2006;133(6):664–666
30. Giannini S, Cecchetti L, Mezzasoma AM, Gresele P. Diagnosis of platelet-type von Willebrand disease by flow cytometry. Haematologica 2010;95(6):1021–1024
31. Franchini M, Montagnana M, Lippi G. Clinical, laboratory and therapeutic aspects of platelet-type von Willebrand disease. Int J Lab Hematol 2008;30(2):91–94
32. Guerrero JA, Kyei M, Russell S, et al. Visualizing the von Willebrand factor/glycoprotein Ib-IX axis with a platelet-type von Willebrand disease mutation. Blood 2009;114(27): 5541–5546

33. Suva LJ, Hartman E, Dilley JD, et al. Platelet dysfunction and a high bone mass phenotype in a murine model of platelet-type von Willebrand disease. Am J Pathol 2008;172(2):430–439
34. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand's disease. Blood 1987;69(2):454–459
35. Favaloro E. Von Willebrand disease: local diagnosis and management of a global issue. Semin Thromb Hemost 2011;37(5):440–455
36. Hamilton A, Ozelo M, Leggo J, et al. Frequency of platelet type versus type 2B von Willebrand disease. An international registry-based study. Thromb Haemost 2011;105(3):501–508