

## Hyperactive GPIb-von Willebrand factor interaction as cause of thrombocytopenia: altered platelet formation versus clearance

Kathleen Freson

Department of Cardiovascular Sciences, Center for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium

E-mail: KATHLEEN FRESON - Kathleen.freson@kuleuven.be

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Platelet-type von Willebrand disease (PT-VWD; MIM177820) is a very rare dominant platelet disorder caused by missense variants in the *GP1BA* gene that codes for glycoprotein Ibalpha (GPIb $\alpha$ ), the receptor for von Willebrand factor (VWF).<sup>1</sup> These variants are gain-of-function (GOF) variants that result in enhanced binding of VWF to platelet GPIb $\alpha$  with subsequent removal of high-molecular-weight forms of VWF in plasma.<sup>2,3</sup> Though this results in hyper-responsive platelets, PT-VWD patients present with mild mucocutaneous bleeding symptoms and intermittent thrombocytopenia with presence of some larger platelets. For years, it was hypothesized that this thrombocytopenia was the result of enhanced platelet clearance from the circulation due to the presence of the VWF-loaded platelets, but this was actually never shown *in vivo*. No previous attempts had been made to study megakaryopoiesis in these patients.

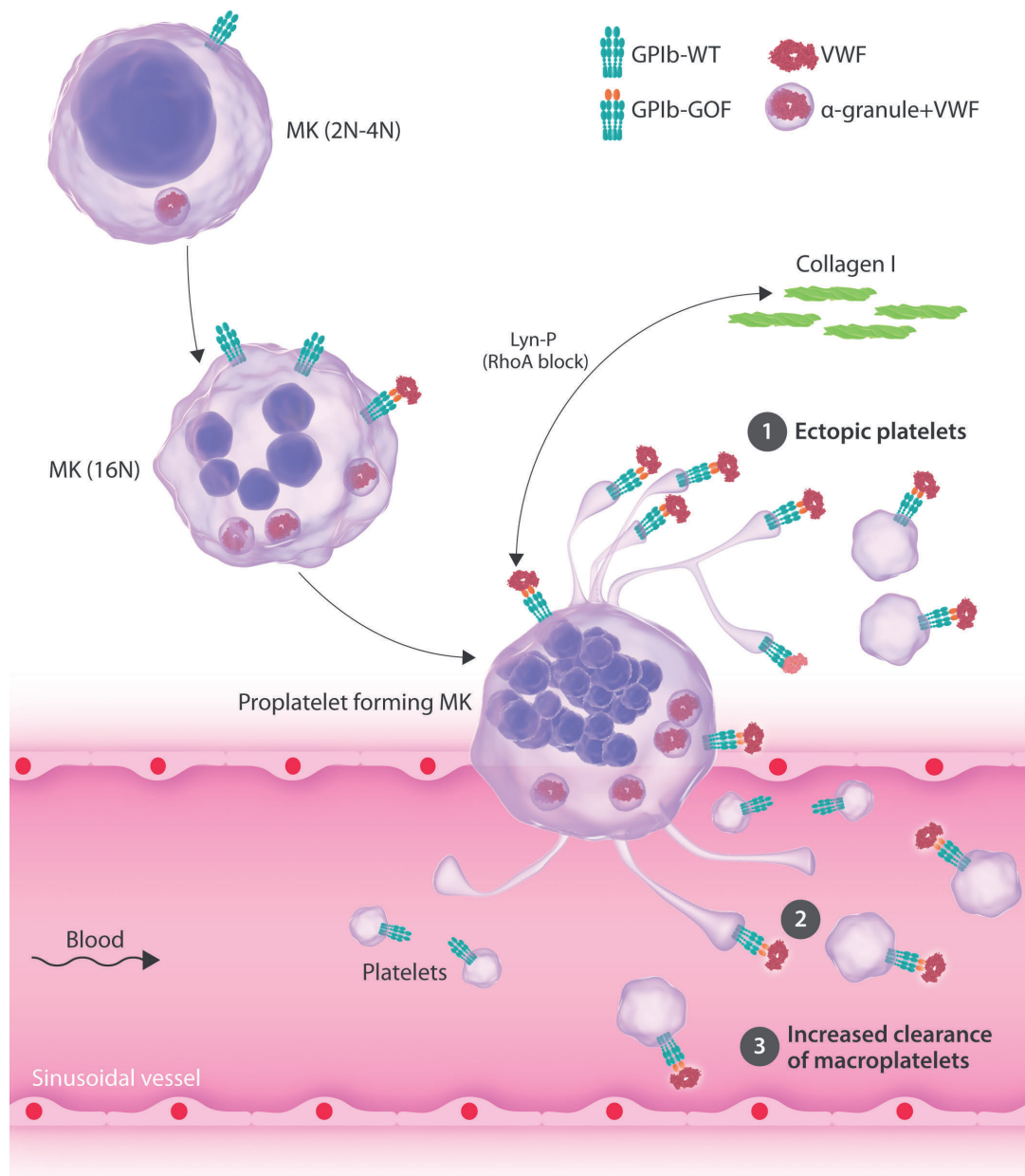
The study by Bury *et al.*, in this issue of the Journal, now provides three rationales that corroborate the clinical and laboratory defects found for PT-VWD by studying megakaryopoiesis and platelet clearance using a human (p.M239V) and mouse (p.G233V) model for this disease (Figure 1).<sup>4</sup> They found that: 1) ectopic platelets are released in the bone marrow; 2) PT-VWD megakaryocytes (MK) release larger but less (pro)platelets; and 3) GPIb-VWF positive platelets are more rapidly released from the blood circulation. These mechanisms support a combined defect in platelet formation and clearance to explain thrombocytopenia in PT-VWD. This study has used CD34<sup>+</sup> hematopoietic stem cells (HSC) from a PT-VWD patient to study *in vitro* megakaryopoiesis. Data supported a mild defect in proplatelet formation with a reduction in the number of proplatelet tips that were larger, though a similar percentage from all MK were able to form proplatelets when compared to those from healthy donors. Interestingly, proplatelet formation was enhanced when PT-VWD MK were spread on collagen (but not on VWF or fibrinogen) and signaling studies revealed that this was due to increased enhanced Lyn phosphorylation (Lyn-P) resulting from the spontaneous GPIb-VWF interaction. Lyn-P blocks the normal RhoA-dependent inhibition of proplatelet formation in the presence of collagen I. Such a defect would result in the ectopic release of platelets in the bone marrow, and histological examination of bone marrow sections from the PT-VWD patient showed the presence of slightly more platelets ( $P < 0.05$ ) when compared to slides from three controls and three immune thrombocytopenia patients. Probably more studies in other PT-VWD patients are required to support these findings, though at least mouse studies using a PT-VWD knock-in model confirmed all these findings. The PT-VWD mouse model was also used to study platelet clearance and a significant reduced platelet half-life was

observed for platelets with mutant GPIb $\alpha$  that captured VWF. Interestingly, desmopressin (DDAVP) administration to PT-VWD mice to increase their plasma VWF levels further decreased their lower platelet count, and this was associated with a further drop in platelet lifespan. PT-VWD mouse platelets did not expose increased levels of phosphatidylserine, excluding an important role for apoptosis as mediator for platelet clearance.

PT-VWD can be misdiagnosed as type 2B von Willebrand disease (VWD2B; MIM613554), as these two platelet disorders have very similar phenotypic parameters and clinical symptoms.<sup>5</sup> VWD2B is caused by dominant GOF variants in the gene coding for von Willebrand factor *VWF*, generating mutant VWF with enhanced affinity for GPIb $\alpha$  with subsequent removal of high-molecular-weight forms of VWF in plasma. VWD2B patients and mice display mild bleeding and macrothrombocytopenia.<sup>6,8</sup> Different studies have already investigated possible mechanisms that could explain the thrombocytopenia present in VWD2B. Briefly, by studying nine unrelated VWD2B patients with different GOF variants,<sup>6,7</sup> electron microscopy showed that their platelets are larger, with the presence of giant platelets and platelet agglutinates. *In vitro* megakaryopoiesis studies using VWD2B HSC showed a reduction in proplatelet formation from enlarged swellings, and MK with a disorganized demarcation membrane system and abnormal granule distribution, pointing to a role of mutant VWF during MK maturation in conditions where the GOF-VWF could only be produced by the MK.<sup>6</sup> When HSC from healthy donors are cultured in conditions where exogenous wild-type (WT) or GOF (p.R1306W) VWF is added to the thrombopoietin containing medium, (pro)platelet-formation was enhanced compared to conditions without VWF, but, remarkably, the GOF-VWF had a more pronounced stimulatory effect compared to WT-VWF. This seems to indicate that the GOF-VWF produced by VWD2B MK act differently to when adding exogenous GOF-VWF to normal MK. Interestingly, treatment of VWD2B MK with exogenous WT-VWF did raise the (pro)platelet counts though these remained lower than the numbers obtained from control MK. *In vitro* megakaryopoiesis studied for PT-VWD was only performed in conditions of endogenous VWF produced by the MK. The role of platelet- versus endothelial cell-derived VWF for *in vivo* megakaryopoiesis in healthy or diseases models has not yet been studied. A similar MK defect was described later for the VWD2B (p.V1316M) knock-in mouse model.<sup>9</sup> HSC from these mice produce less proplatelet-forming MK (in contrast to the PT-VWD mouse model) with decreased numbers of proplatelets per MK that have a larger size (similar to the PT-VWD mouse model). Signaling studies revealed a strong upregulation of the RhoA/LIMK/cofilin pathway that resulted in F-actin accu-

mulation as likely cause of a (pro)platelet formation defect.<sup>8</sup> Interestingly, the proplatelet formation defect present in mouse VWD2B MK could be rescued by treatment with LIMkinase (LIMK) or RhoA kinase (ROCK1) inhibitors.<sup>8</sup> Moreover, the defect in platelet count and size could be rescued in VWD2B mice that were treated with a LIMK inhibitor.<sup>8</sup> This pathway was not studied in PT-VWD models, and it would be very interesting to see if a similar approach could also rescue the thrombocytopenia in PT-VWD mice. The possibility of ectopic platelet release in

VWD2B was not studied in the mouse model, but a bone marrow aspirate in a child with VWD2B revealed the presence of large platelet clumps and megakaryocyte nuclei surrounded by halos of clumped platelets.<sup>9</sup> Further studies should be performed to strengthen this initial finding. Finally, enhanced clearance of VWF-platelet complexes has been suggested to occur in VWD2B.<sup>10</sup> Studies in VWF2B mice have shown the involvement of macrophages in the removal of such VWF-platelet complexes, and significantly more platelets were found in liver and spleen of VWD2B



**Figure 1. Model for platelet-type von Willebrand disease (PT-VWD)-associated thrombocytopenia explains a defect in platelet formation and clearance.** Megakaryocytes (MK) mature in the bone marrow and express von Willebrand factor (VWF) that is typically stored in their alpha ( $\alpha$ ) granules and the glycoprotein Ib (GPIb) receptor. PT-VWD is caused by a gain-of-function (GOF) variant in GPIb $\alpha$  that results in spontaneous binding to VWF. Thrombocytopenia present in PT-VWD seems to result from diverse mechanisms. 1) Ectopic platelets are released in the bone marrow due to enhanced Lyn phosphorylation (Lyn-P) as a result of the GPIb-VWF interaction and Lyn-P blocks the normal RhoA-dependent inhibition of proplatelet formation in the presence of collagen. 2) PT-VWD MK release larger but less (pro)platelets. 3) GPIb-VWF positive platelets are more rapidly released from the blood circulation. WT: wild type.

mice compared with control mice.<sup>11</sup> Moreover, a significantly reduced platelet half-life was observed for VWD2B platelets that have captured mutant VWF.<sup>11</sup> Similar to observations in PT-VWD, this shorter platelet half-life was not due to differences in the apoptotic properties detected in VWD2B platelets.<sup>12</sup> It was recently found that GPIIb $\alpha$  upon binding to active VWF (e.g. VWD2B plasma) under physiological shear stress unfolds its mechanosensory domain near the platelet surface. This then triggers intracellular signaling, with exposure of  $\beta$ -galactose on the platelet surface that could favor platelet clearance *via* its interaction with the Ashwell–Morell receptor.<sup>13</sup> A follow-up study did quantify  $\beta$ -galactose, as a marker for sialic acid removal, on platelets from VWD2B patients and mice and confirmed increased levels.<sup>14</sup> However, treatment of VWD2B mice with sialidase inhibitors was not associated with the recovery of a normal platelet count.<sup>14</sup> Further studies are definitely required to clarify the exact cause of enhanced platelet clearance expected for VWD2B and PT-VWD.

A comparison of studies in PT-VWD *versus* VWD2B that have focused on the cause of thrombocytopenia in these platelet disorders clearly show similarities, but also point to unanswered questions. Platelet formation and clearance defects have been described for both, but it remains unclear if any process is dominant for these diseases. Genetic studies are required to diagnose these disorders.<sup>15</sup>

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