Platelet morphological changes in two patients with von Willebrand disease type 3 caused by large homozygous deletions of the von Willebrand factor gene

by Paquita Nurden, Alan T Nurden, Silvia La Marca, Margherita Punzo, Luciano Baronciani, Augusto B. Federici

Haematologica 2009 [Epub ahead of print]
doi:10.3324/haematol.2009.012658

Publisher’s Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. This paper will now undergo editing, proof correction and final approval by the authors. Please note that during this production process changes may be made, and errors may be identified and corrected. The final version of the manuscript will appear both in the print and the online journal. All legal disclaimers that apply to the journal also pertain to this production process.

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature. Haematologica is the official organ of the European Hematology Association (www.ehaweb.org).

Support Haematologica and Open Access Publishing by becoming a member of the European Hematology Association (EHA) and enjoying the benefits of this membership, which include free participation in the online CME program.
Platelet morphological changes in two patients with von Willebrand disease type 3 caused by large homozygous deletions of the von Willebrand factor gene

Platelet morphological defects have previously been described in von Willebrand disease type 2B (VWD2B), we now describe that they may also occur in patients with VWD3 lacking both platelet and plasma von Willebrand factor (VWF). Electron microscopy (EM) and immunofluorescence labelling (IF) were used to examine platelets from two VWD3 patients with a homozygous deletion involving VWF and TMEM16B genes. Platelet size heterogeneity was seen in both patients, with an unusual characteristic being the presence of a subpopulation of long thin platelets. The additional detection of circulating megakaryocytes and derived fragments suggests that the absence of VWF can affect megakaryocytopoiesis.

VWF is essential to platelet function mediating adhesion and shear-dependent thrombus formation on the vessel wall. Yet relatively little is known about its role in megakaryocytopoiesis. Macrothrombocytopenia is found in about 30% of patients with von Willebrand disease type 2B (VWD2B) and the fall in platelet count can be severe. In some families, circulating platelet agglutinates are present. VWD2B results from mutations in exon 28 of the VWF gene that lead to amino acid substitutions in the VWF A1 domain. The result is a gain-of-function and VWF multimers that spontaneously bind to glycoprotein (GP)Ib on platelets. VWD type 3 (VWD3) is characterized by severely decreased or absent expression of VWF resulting from a variety of mutations or VWF gene deletions that are sometimes accompanied by alloantibody development.

We have previously reported impaired megakaryocytopoiesis due to a precocious interaction between GP Ibα with newly synthesized VWF in MKs of a family with VWD2B given by a R1308P mutation. In vitro studies performed on MKs in culture have confirmed that proplatelet formation is inhibited by blockade of GP Ibα. In continuing our investigations into the importance of VWF for platelet production, we have now examined platelet morphology for 2 patients with VWD3 caused by a previously characterized homozygous 253-kbp deletion involving VWF and TMEM16B. Neither patient possessed detectable VWF:Ag in either their plasma or platelets and their bleeding scores were high (P1, 24; P2, 25). Their platelet counts at the time of study were 241,000/µL (P1) and 149,000/µL (P2) (control range 150,000-300,000/µL).

Electron microscopy (EM) was used to examine platelet morphology. Figure 1 (a–f) shows a wide range of platelet size heterogeneity in both patients. Illustrated are enlarged and sometimes rounded platelets with internal membrane complexes and a heterogeneous α-granule distribution (a,c). Enlarged α-granules were occasionally observed. An unexpected finding was the presence of very long thin structures (c, d) for these have not been reported in VWD2B. The structure in (f) resembles more a MK fragment. In morphometric studies, a minimum of 100 platelet sections of platelets were analyzed for each subject and compared to the results obtained for 4 control donors. Platelet maximal and minimal diameters were measured using the Software Image J (NIH, Bethesda, MD, USA). Statistics were performed using the students t-test or the Pearson χ² test. Results showed that the mean maximal diameter was 3.3±0.8 µm for P1 and 3.5±1.6 µm for P2 (controls 2.7±0.5 µm, p=0.01), while the minimum diameters were 1.4±0.7 µm for P1 and 1.5±0.7 µm for P2 (controls 1.1±0.8 µm, p=0.02). A striking difference was the % of platelets with a diameter greater than 3 µm; for P1 it was 66%, P2 57% and for the controls 24% (p=0.001).

The large size of some of the cells seen in EM prompted us to immunolabel frozen-thin sections with a mixture of AF-2 (anti-αIIbβ3), Bx-1 anti-GPIIbα and FMC25 (anti-GPIX), glycoproteins specific for the platelet and MK lineage (Figure 2A). Cells with a large nucleus surrounded by cytoplasm containing granules were labelled with the platelet-specific antibodies, clearly suggesting the presence of circulating MKs (a). For comparison, a large round platelet from the same patient is shown in (b) while in (c) the structure resembles a detached MK frag-
and the surrounding cytoplasm is very thin and irregular but different size positive for plex and DAP (nucleus). Results for VWD3 show cells of widely different immunogold labelling with a mixture of anti-αIIb/3 and anti-GPIba MoAbs of cells obtained from P1. A large multilobulated nucleus (N) surrounded by cytoplasm with α-granules is shown in (a). Intracellular and surface labelling (arrow heads) for platelet GPIba MoAbs of cells obtained from P1. A large multilobulated (a) while nuclei surrounded by remnant portions of cytoplasm strongly positive for αIIb/3 complex and DAP (nucleus). Results for VWD3 show cells of widely different size positive for αIIb/3. In (b, c) the cells contain a nucleus and the surrounding cytoplasm is very thin and irregular but heavily labelled. Control platelets are shown for comparison in (d).

Figure 2. Circulating MKs in VWD3. A/ Electron micrographs showing immunogold labelling with a mixture of anti-αIIb/3 and anti-GPIba MoAbs of cells obtained from P1. A large multilobulated nucleus (N) surrounded by cytoplasm with α-granules is shown in (a). Intracellular and surface labelling (arrow heads) for platelet markers indicate that this cell belongs to the megakaryocytic lineage. Labelling of a large round platelet (b), and a tentatively identified cytoplasmic MK fragment (panel c), is also shown. Bars=1 μm B/ Immunofluorescence labelling of blood smears from the VWD3 patients using the MoAb AP-2 specific for the αIIb/3 complex and DAP (nucleus). Results for VWD3 show cells of widely different size positive for αIIb/3. In (b, c) the cells contain a nucleus and the surrounding cytoplasm is very thin and irregular but heavily labelled. Control platelets are shown for comparison in (d).

References