vant role.

In conclusion, in a large cohort of thalassemia and sickle cell patients the use of gadopentate dimeglumine and gadobutrolo in CMR seems to be safe and well tolerated, with a risk comparable to the general population. These data support the routine use of Gd chelates contrast agents in MR and especially in CMR to detect myocardial fibrosis/necrosis for diagnostic and clinical management of thalassemia or sickle cell patients. However, gadolinium should be used with caution in patients with hemoglobinopathies who have severe renal dysfunction.

Antonella Meloni,[1] Brunella Favilli,[1] Vincenzo Positano,[1] Paolo Cianciulli,[2] Aldo Filosa,[3] Antonella Quarta,[4] Domenico D'Ascola,[5] Gennaro Restaino,[6] Massimo Lombardi,[1] and Alessia Pepe [1]

[1]MRI Laboratory, "G. Monasterio" Foundation, Institute of Clinical Physiology, CNR, Pisa; [2]Centro Talassemie, "Sant'Eugenio" Hospital, Roma; [3]UOS Thalassemia, Cardarelli Hospital, Napoli; [4]A. Perrino Hospital, Brindisi; [5]U.O. Microcitemie A.O. "Bianchi-Melacrino-Morelli", Reggio Calabria, and [6]Radiology Department, "John Paul II" Catholic University, Campobasso, Italy

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Correspondence: Antonella Meloni, MRI Laboratory, "G. Monasterio" Foundation, Institute of Clinical Physiology, CNR, Via Moruzzi, 1, 56124 Pisa, Italy.
E-mail: antonella.meloni@ifc.cnr.it

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Platelet morphological changes in 2 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 labeling (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.[1] 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.[2] In some families, circulating platelet agglutinates are present.[2-5] 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.[1,6,7]

We have previously reported impaired megakaryocytopoiesis due to a precocious interaction between GPIbα with newly synthesized VWF in MKs of a family with VWD2B given by a R1308P mutation.[3] *In vitro* studies performed on MKs in culture have confirmed that proplatelet formation is inhibited by blockade of GPIb.[8] 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*.[7] Neither patient possessed detectable VWF:Ag in either their plasma or platelets and their bleeding scores[2] were high (P1, 24; P2, 25). Their platelet counts at the time of study were 241×10^9/L (P1) and 149×10^9/L (P2) (control range $150\text{-}300\times10^9$/L).

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,e). Enlarged α-granules were occasionally observed. An unexpected finding was the presence of very long thin structures (c, d) as these have not been reported in VWD2B.[2] 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 Student's *t* test or Pearson's χ^2 test. Results showed that

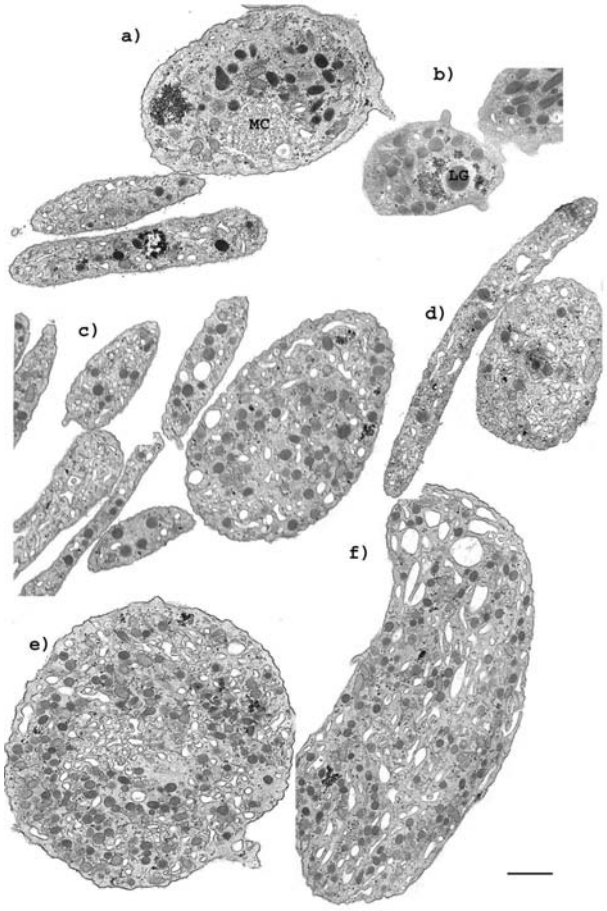

Figure 1. Platelet morphology in VWD3. A gallery of electron micrographs (EM) of platelets from P1 and P2. EM was performed according to our standard procedures.[3] Note the presence of elongated and sometimes very thin platelets. Nevertheless, size heterogeneity is considerable, and a round platelet with membrane complexes (MC) is shown in (a) while a large granule (LG) is to be seen in (b). A large round platelet is shown in (e); whereas a fragment probably abnormally detached from an MK is shown in (f). Bar=1 μm

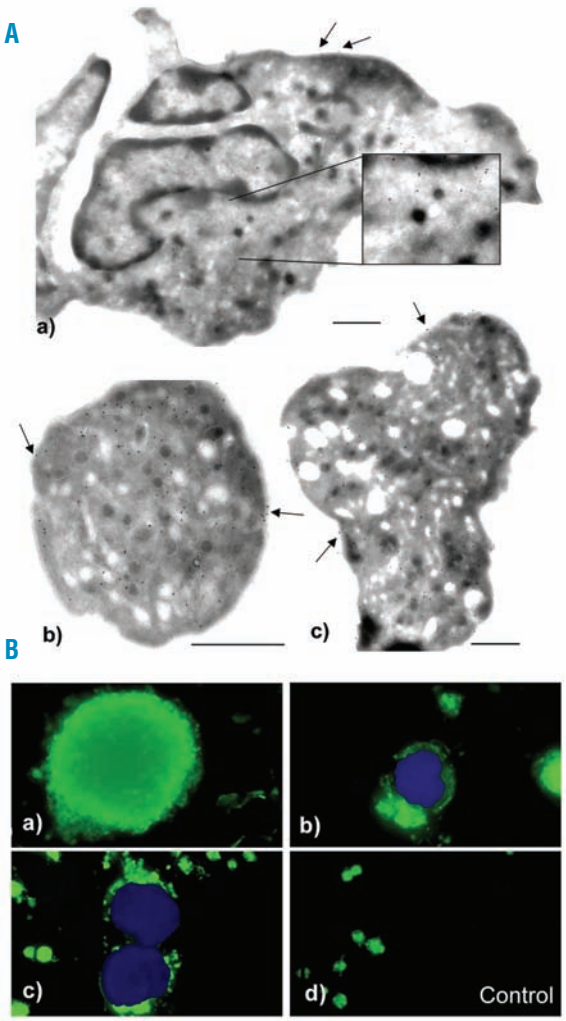

Figure 2. Circulating MKs in VWD3. (A) Electron micrographs showing immunogold labeling with a mixture of anti-αIIbβ3 and anti-GPIba MoAbs of cells obtained from P1. A large multilobulated nucleus (N) surrounded by cytoplasm with α-granules is shown in (a). Intracellular and surface labeling (arrow heads) for platelet markers indicate that this cell belongs to the megakaryocytic lineage. Labeling of a large round platelet (b), and a tentatively identified cytoplasmic MK fragment (panel c), is also shown. Bars=1 μm. (B) Immunofluorescence labeling of blood smears from the VWD3 patients using the MoAb AP-2 specific for the αIIbβ3 complex and DAP (nucleus). Results for VWD3 show cells of widely different size positive for αIIbβ3. In (b, c) the cells contain a nucleus and the surrounding cytoplasm is very thin and irregular but heavily labeled. Control platelets are shown for comparison in (d).

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 percentage 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[9,10] with a mixture of AP-2 (anti-αIIbβ3), Bx-1 anti-GPIbα 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 labeled 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 fragment; both are labeled by the anti-platelet antibodies. Immunofluorescence labeling and confocal microscopy was performed with the same MoAbs as described.[11] Blood smears of the patients with VWD3 were compared to those of controls. Figure 2B shows for P1 what may be a MK fragment (a) while nuclei surrounded by remnant portions of cytoplasm strongly positive for αIIbβ3 were also seen (b, c). For the other patient with VWD3, size heterogeneity was observed but nucleated αIIbβ3-labeled cells were found less frequently (*data not shown*).

We have shown for the first time that platelet size and morphology may also be abnormal in VWD3. These results suggest that a complete absence of VWF (VWD3), as well as enhanced VWF binding to GPIb (VWD2B), may interfere with megakaryocytopoiesis. It is tempting to suggest that in VWD3 some MKs fail to follow a normal maturation or migration. The presence of very long thin platelets suggests that the absence of VWF results in modifications in the endgame of proplatelet formation and platelet release. Nevertheless, the absence of VWF does not give rise to a Bernard-Soulier-like giant platelet

syndrome.[9] Conceivably, the absence of VWF in VWD3 could result in the loss of a GPIb-dependent signaling pathway that helps regulate MK maturation and/or proplatelet formation. While the platelet changes shown in the VWD3 patients do not give rise to major thrombocytopenia, and as such may not add to the clinical phenotype of VWD3, they may intervene under stress conditions such as during major bleeding when megakaryocytopoiesis is stimulated. It is to be emphasized that our 2 patients belong to a select subpopulation of VWD3 and that the gene deletion extends to *TMEM16B* that encodes a Ca^{2+}-activated Cl^- channel.[12] While it is not currently known if this gene is expressed in the MK-lineage, further studies are merited on a much larger number of VWD3 patients arising from a range of genetic defects to define the role of VWF in finely regulating platelet production.

Paquita Nurden,[1] Alan T Nurden,[1] Silvia La Marca,[2] Margherita Punzo,[2] Luciano Baronciani,[2] and Augusto B. Federici[2]

[1]Centre de Référence des Pathologies Plaquettaires, Plateforme Technologique et d'Innovation Biomédicale, Hôpital Xavier Arnozan, Pessac, France; [2]Angelo Bianchi Bonomi Hemophilia Thrombosis Center, Department of Medicine and Medical Specialities, IRCCS Maggiore Hospital, Mangiagalli, Regina Elena Foundation and University of Milan, Milan, Italy.

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Correspondence: Paquita Nurden MD, Centre de Référence des Pathologies Plaquettaires, Plateforme Technologique et d'Innovation Biomédicale, Hôpital Xavier Arnozan, 33600 Pessac, France. E-mail: paquita.nurden@cnrshl.u-bordeaux2.fr

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