Journal of Thrombosis and Haemostasis, **17**: 795–802 DOI: 10.1111/jth.14424

BRIEF REPORT

First description of an IgM monoclonal antibody causing $\alpha_{IIb}\beta_3$ integrin activation and acquired Glanzmann thrombasthenia associated with macrothrombocytopenia

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To cite this article: Pillois X, Guy A, Choquet É, James C, Tuffigo M, Viallard J-F, Garcia C, Bordet J-C, Jandrot-Perrus M, Payrastre B, Fiore M. First description of an IgM monoclonal antibody causing $\alpha_{IIb}\beta_3$ integrin activation and acquired Glanzmann thrombasthenia associated with macrothrombocytopenia. *J Thromb Haemost* 2019; **17**: 795–802.

Essentials

- Acquired Glanzmann thrombasthenia (GT) is generally caused by anti-$\alpha_{IIb}\beta_3$ autoantibodies.
- We report the case of a man with an acquired GT phenotype associated with macrothrombocytopenia.
- Perturbed platelet function were associated with an activating anti-$\alpha_{IIb}\beta_3$ IgM autoantibody.
- This novel clinical entity raises interesting questions about the $\alpha_{IIb}\beta_3$ integrin signaling.

Summary. *Background:* Acquired Glanzmann thrombasthenia (GT) is a bleeding disorder generally caused by anti-$\alpha_{IIb}\beta_3$ autoantibodies. *Objectives:* We aimed to characterize the molecular mechanism leading to a progressive GT-like phenotype in a patient with chronic immune thrombocytopenia. *Patient, Methods, and Results:* The patient suffered from repeated episodes of gastrointestinal bleeding; further studies indicated a moderate platelet aggregation defect. A few months later, platelet function showed abolished aggregation using all agonists, but normal agglutination with ristocetin. No platelet-bound antibodies were detected, but the presence of large amounts of an IgM type antibody detected together with $\alpha_{IIb}\beta_3$ in the patient permeabilized platelets suggested that this IgM was an autoantibody causing the internalization of the complex. This was confirmed by the fact that the patient IgM bound to normal platelets but not to platelets from GT type I patients. Moreover, patient′s plasma activated $\alpha_{IIb}\beta_3$ on controls' platelets as evidenced by increased PAC-1 binding. We also demonstrated that the patient plasma triggered $\alpha_{IIb}\beta_3$ outside-in signaling, as β_3 Tyr773 and FAK were phosphorylated, and increased the rate of actin polymerization in resting platelets reflecting an impairment of cytoskeletal reorganization. Because different signs of dysmegakaryopoiesis were also observed in our patient, we evaluated the ability of its serum to impair proplatelets formation and showed that it significantly decreased the number of proplatelet-bearing megakaryocytes in controls' bone marrow stem cells culture compared with normal serum. *Conclusions:* We present the case of a patient with a progressive and severely perturbed platelet function associated with the presence of an IgM activating autoantibody directed against $\alpha_{IIb}\beta_3$.

Keywords: $\alpha_{IIb}\beta_3$ integrin; Glanzmann thrombasthenia; immune thrombocytopenia; macrothrombocytopenia; outside-in signalling.

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Received: 3 November 2018
Manuscript handled by: Y. Ozaki
Final decision: F. R. Rosendaal, 28 February 2019

Introduction

Among the wide number of transmembrane receptors expressed at the platelet surface, $\alpha_{IIb}\beta_3$ integrin is the major one [1]. Glanzmann thrombasthenia (GT) is a rare

autosomal recessive bleeding disorder caused by inherited defects of the $\alpha_{IIb}\beta_3$ integrin [2]. Classically, in GT, platelets fail to bind fibrinogen (Fg) and do not aggregate in response to all physiological stimuli. Platelet counts are usually normal in patients with $\alpha_{IIb}\beta_3$ deficiency, suggesting a modest role of the integrin in platelet production and morphology. In rare patients, however, gain-of-function mutations in either *ITGA2B* or *ITGB3* can interfere with megakaryopoiesis, leading to variant forms of GT with platelet dysfunction and macrothrombocytopenia [3–6]. These mutations cause constitutive activation of the integrin locked in a high affinity state. Recent works from Bury *et al*. demonstrated that such permanent triggering of $\alpha_{IIb}\beta_3$-mediated outside-in signaling leads to integrin internalization and impaired cytoskeletal remodeling [6,7].

On the other hand, acquired GT associated with autoantibodies directed against $\alpha_{IIb}\beta_3$ has been also reported [8]. Our study concerns the first description of a monoclonal anti-$\alpha_{IIb}\beta_3$ IgM autoantibody causing a phenotype quite similar to what can be seen with germline mutations causing constitutive $\alpha_{IIb}\beta_3$-mediated outside-in signaling.

Material and methods

Case report

The patient has been previously reported by us as a 53-year-old man with chronic refractory immune thrombocytopenia diagnosed in 2003 and treated with romiplostim (Amgen, Mississauga, Canada) since 2010 [9]. In July 2013, in spite of subnormal platelet counts, he suffered from severe and repeated episodes of gastrointestinal bleeding. He was then referred to our center, where a battery of hemostasis tests was performed on several occasions.

Blood samples

All individuals gave written informed consent that complied with our local ethics committee and the Helsinki Declaration. Blood samples were obtained from the patient, healthy volunteers, and from patients with inherited platelet disorders. The demonstration of the presence of a circulating antibody interfering with platelet activation in the patient plasma was obtained by mixing tests using different platelet function assays.

Monoclonal antibodies (MoAbs)

SZ2 (anti-GPIbα) and CD61 (anti-β_3) were purchased, respectively, as isolated IgG from Beckman Coulter and Dako (Glostrup, Denmark). PAC-1 (an IgM recognizing activated but unoccupied $\alpha_{IIb}\beta_3$ complexes) was purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Fluorescein isothiocyanate (FITC)-labeled antihuman IgG, IgM, or IgA were from Jackson ImmunoResearch (West Grove, PA, USA).

Platelet aggregation testing

Platelet aggregation was tested in citrated platelet-rich plasma (PRP) using 1.2 mg mL^{-1} ristocetin (Stago, Asnières-sur-Seine, France), 10 μM ADP (Calbiochem, San Diego, USA), 1 mM arachidonic acid (Nu Chek Prep, Elysian, MN, USA), 50 μM thrombin receptor activating peptide (TRAP-14; Neosystem SA, Strasbourg, France), and 2 μg mL^{-1} Horm equine tendon collagen (Nycomed Pharma, Unterschleibheim, Germany) in an APACT 4004 aggregometer (Elitech, Salon de Provence, France) according to standard procedures [10].

Platelet flow cytometry

Surface expression of $\alpha_{IIb}\beta_3$ and GPIb were measured in PRP using a calibrator kit (Biocytex, Marseille, France). Analysis of platelet surface antibodies was performed on platelets incubated for 30 min at room temperature with different antihuman Ig antibodies. Fibrinogen binding was measured on PRP incubated with Alexa Fluor 488–labeled human fibrinogen (Molecular Probes, Eugene, OR, USA). Platelets were analyzed in a Cytomics FC500 flow cytometer (Beckman Coulter, Villepinte, France) and results were expressed as mean fluorescence intensity.

Confocal microscopy

Smears of citrated blood were prepared and fixed with cold acetone, washed in phosphate buffered saline 0.5% albumin, and then incubated for 1 h after simultaneous addition of predetermined optimal dilutions of MoAbs against $\alpha_{IIb}\beta_3$ or GPIbα, and a polyclonal rabbit anti-von Willebrand factor antibody. $\alpha_{IIb}\beta_3$ and GPIbα were detected using a species-specific polyclonal antibody to mouse IgG conjugated to Alexa-Fluor 568 (Thermo Fisher Scientific, Waltham, MA, USA), and von Willebrand factor with an FITC-conjugated goat antirabbit IgG antibody. The slides were mounted using Fluoroshield with 4,6-diamidino-2-phenylindole (Sigma-Aldrich, Saint-Louis, Missouri, USA) and examined with a Carl Zeiss LSM700 confocal microscope (Zeiss, Oberkochen, Germany) equipped with a 63× Plan Apochromat 1.4 NA objective. Maximum intensity projection images and intensity profile measurements were performed using FIJI software [26].

Actin polymerization

PRP were stimulated with ADP (20 μM) or TRAP (50 μM) for 15 min at 37 °C, fixed with CellFIX (Becton

Dickinson, Franklin Lakes, NJ, USA), permeabilized with 0.4% Triton, stained with FITC-conjugated Phalloidin and analyzed by flow cytometry.

Western blotting

Washed platelets from the patient or control donors were lysed in buffer containing 2% sodium dodecyl sulfate according to our standard procedures. Sodium dodecyl-sulfate–polyacrylamide gel electrophoresis was performed using 10% homogeneous gels and transferred to nitrocellulose membrane. Membranes were probed with an anti-phospho-β_3 (Tyr773) and an anti-β_3 MoAb (Santa Cruz Biotechnology, Dallas, TX, USA), as well as an anti-phospho–FAK (Tyr 397) and an anti-FAK MoAb (Cell Signaling Technologies, Danvers, MD, USA). Bound antibodies were assessed by chemiluminescence using peroxidase-linked antibodies.

Proplatelet formation study

Bone marrow was obtained from patients with normal blood counts undergoing cardiovascular surgery for valve replacement or spine surgery. Immunomagnetic $CD34^+$ cells were selected with the EasySep® kit (STEMCELL, Grenoble, France) according to the manufacturer's protocol. $CD34^+$ cells were seeded in StemSpan SFEM II (STEMCELL) with 100 ng/mL of TPO (human TPO, PeProTech, Rocky Hill, NJ, USA), SCF, Flt-3L, and interleukin-3 at a final concentration of 50 ng mL^{-1} (STEMCELL). Fresh medium with 50 ng mL^{-1} of TPO was added at days 3 and 8. On day 9, megakaryocytes (MKs) were seeded onto 96-well plates in 100 μL of StemSpan SFEM II per well with 50 ng mL^{-1} of TPO. After 9 days of culture, patient and control sera were added every day to each well at 1 : 10 dilution. At the same time, proplatelet-forming and non-proplatelet-forming MKs were counted with a Nikon Eclipse Ti inverted microscope system (Nikon Corp., Tokyo, Japan).

Statistical analysis

To account for variability in experimental conditions among measurements, control and patient samples were handled in parallel when possible. Comparisons between patients and controls were performed with the Mann–Whitney test as appropriate and P values of < 0.05 were considered significant.

Results and discussion

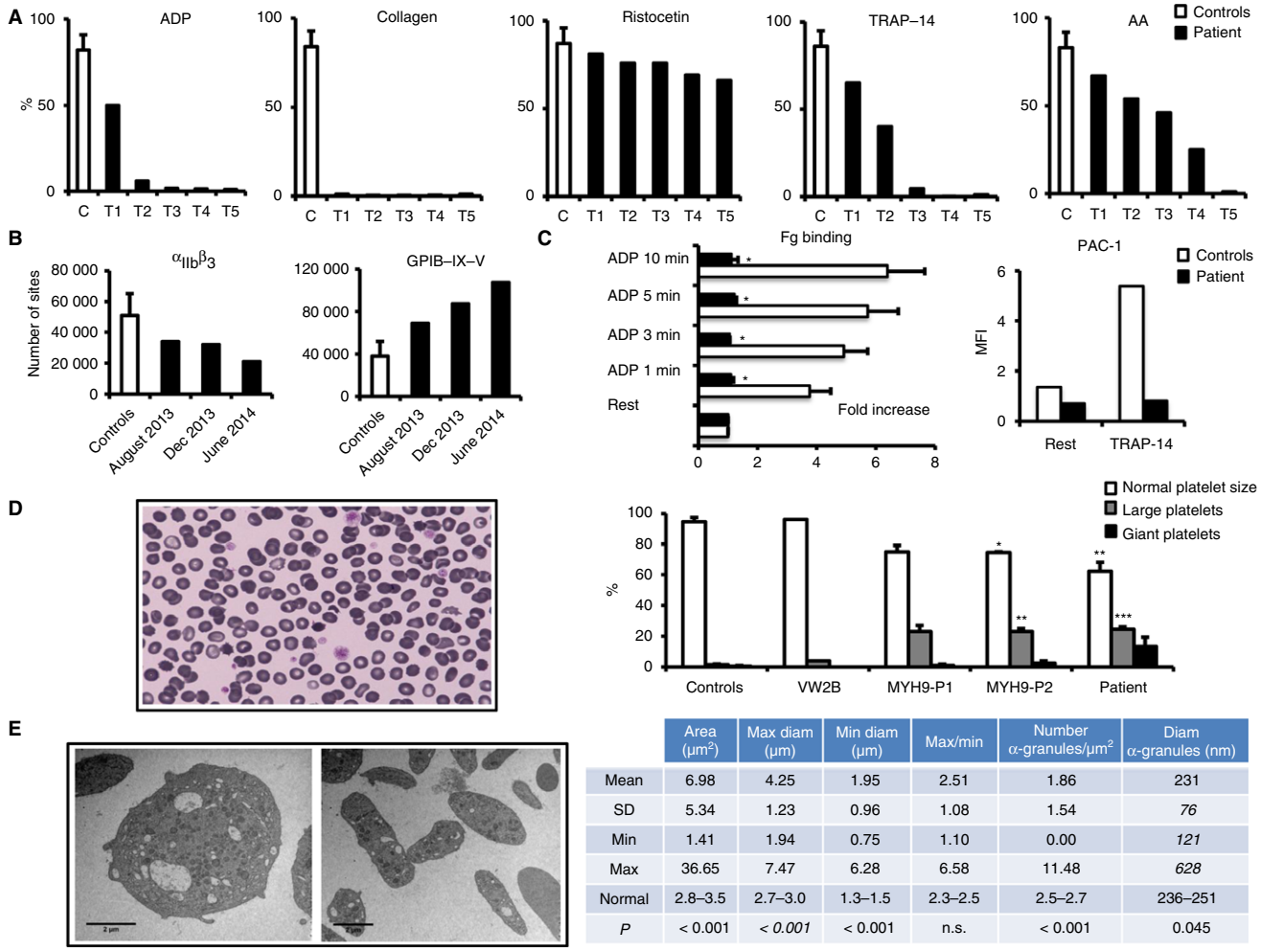
The patient initially presented with a moderate platelet aggregation defect (Fig. 1A). Five months later, platelet aggregation was much reduced using all agonists, whereas agglutination with ristocetin was normal, evoking an acquired GT-like phenotype. Expression of $\alpha_{IIb}\beta_3$ and GPIbα was also studied and confirmed an evolution of the platelet defect in the course of time (Fig. 1B). The $\alpha_{IIb}\beta_3$ complex was present in lesser amounts, whereas the expression of GPIbα progressively increased. Additionally, when stimulated by ADP (10 μM) or TRAP-14 (50 μM), the patient platelets did not bind fluorescent Fg or PAC-1 (Fig. 1C).

Blood smears stained with May–Grünwald–Giemsa and electron microscopy studies showed marked platelet anisocytosis including numerous giant platelets (Fig. 1D,E). These large platelets could account for the apparently increased expression of GPIb.

Sequencing of genomic DNA revealed no potential pathological mutations in the *ITGA2B* and *ITGB3* genes (data not shown). Then, we hypothesized that an autoantibody directed against $\alpha_{IIb}\beta_3$ might cause the platelet defects, but no platelet-bound antibodies could be detected. Massé *et al.* [11] demonstrated that abciximab, the blocking chimeric antibody fragment that targets $\alpha_{IIb}\beta_3$ receptor, may bind to MK plasma membrane and be secondarily internalized; thus, we tested whether a patient's autoantibody would follow a similar process. As shown in Fig. 2A, we demonstrated, using flow cytometry, that permeabilized patient platelets contained large amounts of an IgM type antibody. Consistent with this result, we observed the presence of a 75-kDa band in western blot in reducing conditions corresponding to IgM subunit (data not shown). Moreover, a monoclonal spike (2 g L^{-1}) in the patient's serum was also identified as an IgM kappa immunoglobulin, suggesting that the patient could have developed a monoclonal anti-$\alpha_{IIb}\beta_3$ IgM type antibody.

To test this hypothesis, mixing experiments were performed by adding plasma from the patient to washed control platelets. Following incubation with specific anti-human IgM antibody, control platelets were strongly positive, although mean fluorescence intensity decreased through time (Fig. 2B). Conversely, after prolonged incubation, an IgM antibody was detected in permeabilized platelets, suggesting that this immunoglobulin was secondarily internalized. Moreover's, using confocal microscopy, we observed in the patient platelets, that $\alpha_{IIb}\beta_3$ predominantly localized in the cytoplasm rather than at the platelet surface, suggesting that the IgM antibody reduced the surface expression of $\alpha_{IIb}\beta_3$ by a co-internalization process (Fig. 2D).

To demonstrate that this antibody was directed against $\alpha_{IIb}\beta_3$, washed platelets from GT type I patients were incubated with the patient plasma. The IgM did not bound to GT platelets, confirming that $\alpha_{IIb}\beta_3$ was the target of this antibody (Fig. 2C). To analyze the effect on platelet activation, we investigated the binding of PAC-1 by flow cytometry (Fig. 3A). In these conditions, control platelets were rapidly activated, as indicated by increased binding of PAC-1 in the presence of the patient plasma compared with control plasma. Experiments were also



	Area (μm^2)	Max diam (μm)	Min diam (μm)	Max/min	Number α-granules/μm^2	Diam α-granules (nm)
Mean	6.98	4.25	1.95	2.51	1.86	231
SD	5.34	1.23	0.96	1.08	1.54	*76*
Min	1.41	1.94	0.75	1.10	0.00	*121*
Max	36.65	7.47	6.28	6.58	11.48	*628*
Normal	2.8–3.5	2.7–3.0	1.3–1.5	2.3–2.5	2.5–2.7	236–251
P	< 0.001	*< 0.001*	< 0.001	n.s.	< 0.001	0.045

Fig. 1. Patient platelet function and morphology. (A) Maximal aggregation responses (%) from the patient (filled bars) compared with healthy volunteers (open bars; $n = 30$) induced by 10 μM ADP, 2 μg mL^{-1} collagen, 1.2 mg mL^{-1} ristocetin, 50 μM TRAP-14, and 1 mM arachidonic acid (AA) tested on different occasions. C = controls; T_1 = 30 July 2013; T_2 = 3 December 2013; T_3 = 9 December 2013; T_4 = 25 June 2014; T_5 = 28 August 2015. (B) Evolution of $\alpha_{IIb}\beta_3$ integrin and GPIb-IX-V complex expression on resting platelets from the patient measured by flow cytometry. Results are expressed as "Number of sites" (Reference values, represented as open bars, were provided by the manufacturer). (C) Fibrinogen ($n = 2$; Dates = 2 January 2014 and 13 September 2016) and PAC-1 ($n = 1$; date = 2 January 2014) binding measured by flow cytometry in the patient and different day controls. Activation was obtained by incubation with ADP 10 μM (at different time points = minutes-min.) or TRAP-14 50 μM. (D) Representative image of a patient's peripheral blood smear stained with May–Grunwald–Giemsa showing platelet anisocytosis. The histogram represents the distribution of platelet sizes measured by optical microscopy on blood films stained with May–Grunwald–Giemsa in the patient ($n = 5$; blood samples between 3 December 2013 and 31 March 2015), patients with inherited macrothrombocytopenia (VW2B: von Willebrand type 2B, $n = 1$; MYH9: MYH9 related-disease, $n = 2$), and controls ($n = 2$). A total of 100 platelets were evaluated for each blood smear. Values are expressed as percentages ± standard deviation. Giant platelets were defined as platelets that are, at least, the size of a red blood cell, and platelets larger than half the diameter of red cells were defined as large platelets. (E) Ultrastructural images of the patient platelets (26 July 2017) studied by electron microscopy (EM) showing heterogeneity in platelet size and shape, presence of giant platelets, and heterogeneous α-granule distribution. A table also shows the different platelet characteristics (100 platelets were evaluated for each parameter). Normal values are expressed as mean ± standard deviation. Standard EM was performed using venous blood taken in ACD-A and prepared as previously described (Fiore M, *Platelets*, 2016 Sep;27(6):555–62). (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. controls).

performed by adding a 1-h incubation at 37 °C. We then noted that PAC-1 binding decreased, which could be explained by $\alpha_{IIb}\beta_3$ internalization. We next assessed whether the patient plasma was able to trigger $\alpha_{IIb}\beta_3$ outside-in signaling by measuring the tyrosine phosphorylation of the β_3 subunit and the phosphorylation of FAK. In unstimulated patient platelets, β_3 and FAK were both phosphorylated (Fig. 3B). Similarly, we observed an increased level of integrin β_3 Tyr773 and FAK phosphorylation in washed controls' platelets incubated with the patient plasma. Strikingly, this phenotype resembles that of patients with inherited $\alpha_{IIb}\beta_3$ activating mutations in which outside-in signaling is constitutively activated [3,4].

Bury *et al.* have previously shown that patient platelets with constitutive integrin activation have an increased F-actin content under resting conditions, suggesting that

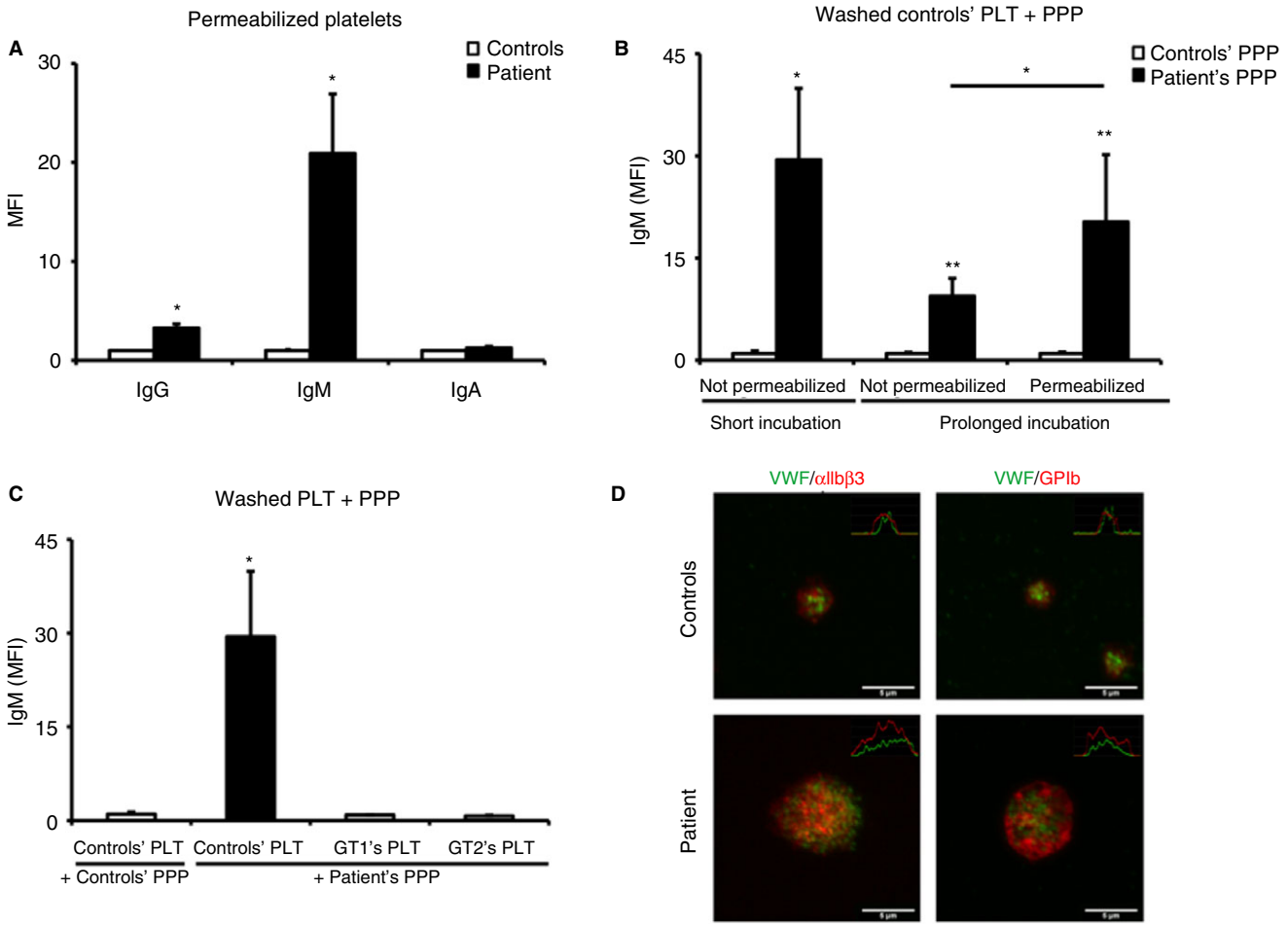


Figure 2. Presence of an internalized anti-$\alpha_{IIb}\beta_3$ IgM antibody. (A) Washed platelets from the patient (dates: 13 November 2015 and 26 February 2016) or controls were permeabilized and incubated at room temperature with FITC-labeled anti-human IgG, IgM or IgA. Results show the strong presence of an IgM antibody in patient platelets ($n = 4$; $^*P < 0.05$ vs. controls). (B) Washed platelets from normal donors were incubated for 30 min at room temperature (short incubation) with control or patient PPP (blood samples collected in August 2015 and 2016) and FITC-labeled anti-human IgM. Mixing experiments were also performed by adding PPP to washed control platelets for one hour at 37 °C (prolonged incubation). Platelets were then permeabilized or not, and incubated for 30 min with FITC-labeled anti-human IgM ($n = 5$; $^*P < 0.05$, $^{**}P < 0.01$ vs. controls). (C) Washed platelets from patients with type I GT or normal donors were incubated for 30 min at room temperature with PPP from the patient or control donors and FITC-labeled anti-human IgM ($n = 5$); GT_1/GT_2: washed platelets from two patients with type I GT mixed with the patient PPP ($n = 3$). $^*P < 0.05$ vs. controls. (D) Two-color confocal microscopy comparing the distribution of antibodies detecting $\alpha_{IIb}\beta_3$ or GPIb (red fluorescence) and von Willebrand factor (green fluorescence; present in α-granule) in fixed and permeabilized platelets. Graphics report the intensity of the fluorescence signal along the *x* axis for each fluorochrome on the optical section. As von Willebrand factor, $\alpha_{IIb}\beta_3$ predominantly localized in the cytoplasm of the patient platelets, whereas GPIb was present at the platelet surface with a distribution quite similar to healthy controls.

permanently triggered outside-in signaling favors actin polymerization [7]. Here, we also show that F-actin content was significantly higher in unstimulated patients' platelets (Fig. 3C). Stimulation with ADP did not significantly increase F-actin content of patient platelets, whereas it doubled it in control platelets. This phenotype was reproduced when washed controls' platelets were incubated with the patient plasma. However, this result was quite surprising because PAC-1 binding was not reduced when platelets from healthy controls were incubated with the patient plasma. Nevertheless, the consequent permanent triggering of $\alpha_{IIb}\beta_3$-mediated outside-in signaling might be only observed after several days of incubation, as it is the case, *in vivo,* with the patient's MKs.

Finally, as different signs of dysmegakaryopoiesis were also observed in our patient, we evaluated the ability of its serum to impair proplatelets formation. For that, number of proplatelet-forming and non–proplatelet-forming MKs were counted under a phase-contrast microscope every 24 h from days 10 to 13. The percentages of proplatelet-forming MKs in presence of the patient's or controls' serum were respectively, 1.6% vs. 5.9% ($P = 0.026$) on day 11, 4.5% vs. 12% ($P = 0.0011$) on day 12, and 8.3% vs. 29.4% ($P < 0.0001$) on day 13 (Fig. 3D). These data indicate that the patient's

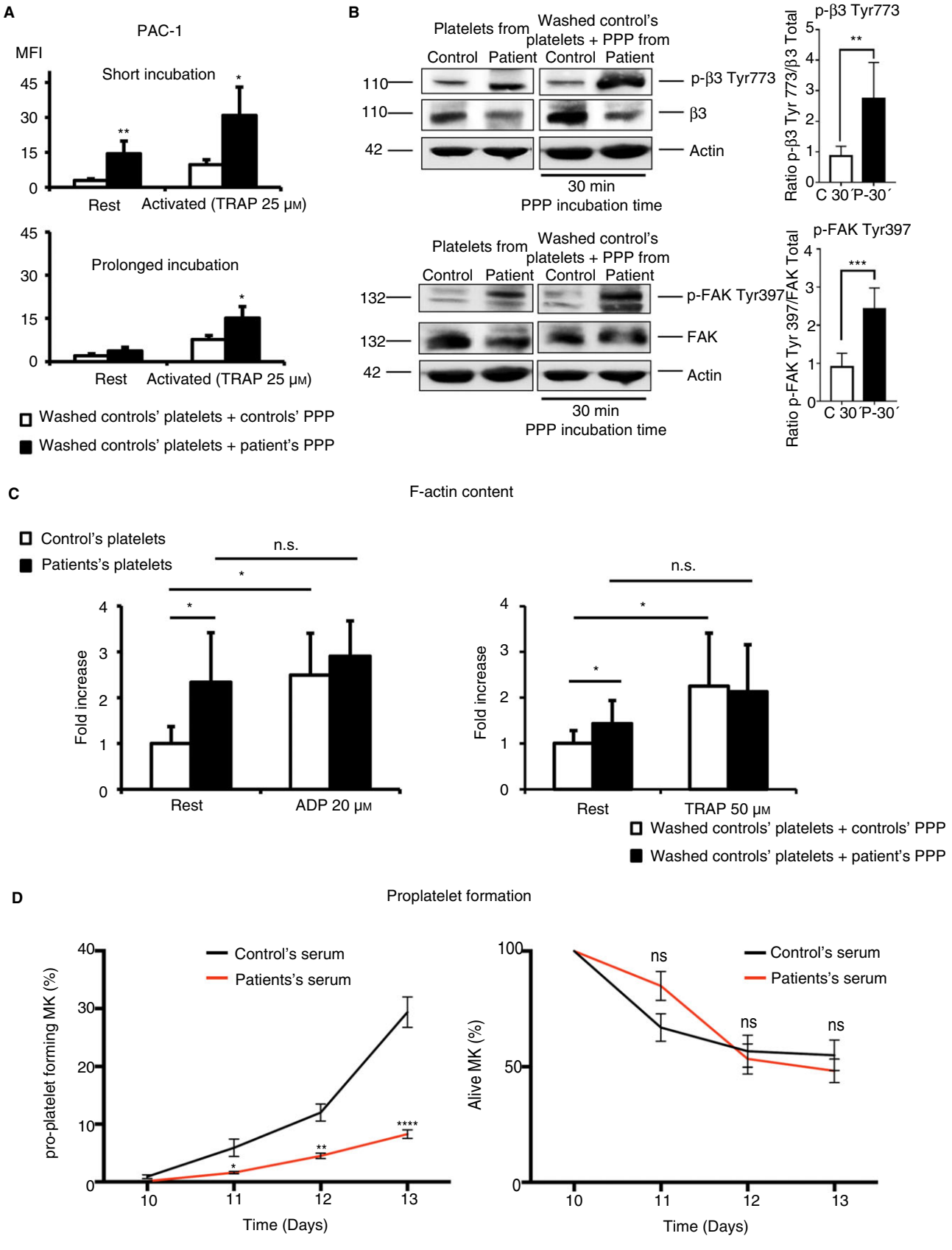


Figure 3. $\alpha_{IIb}\beta_3$ activation and proplatelet formation. (A) Washed platelets from normal donors were incubated for 30 min at room temperature with PPP from the patient (blood samples collected in August 2015 and 2016) or controls and labeled with PAC-1 MoAb ($n = 5$; $^*P < 0.05$, $^{**}P < 0.01$ vs. control). Experiments were also realized by adding 1 h incubation at 37 °C (prolonged incubation). (B) β_3Tyr773 and FAK phosphorylation of control and patient platelets or washed control platelets incubated with the patient or controls' plasma. Results shown for "Platelets" are representative of 2 independent experiments, whereas histograms represent the semiquantitation of each protein for "washed control's platelets + PPP".$^{**}P < 0.01$ and $^{***}P < 0.001$. The results are representative of four distinct experiments. (C) Polymerized actin (F-actin) content of controls and patient platelets (collected between February and March 2018) measured by flow cytometry before and after stimulation with ADP 20 μM; $n = 5$. Experiments were also reproduced by mixing washed control platelets with PPP from the patient (collected between December 2013 and 2014) or donors; $n = P < 0.05$). (D) Cultured megakaryocytes (MKs) were treated with either the patient or control serum and number of proplatelet-forming and non-proplatelet-forming MKs were counted under a phase-contrast microscope every 24 h from days 10 to 13. Curves of the percentages of viable MKs and analysis of proplatelet release expressed as the ratio of proplatelet-forming MKs/total number of MKs per well in a 96-well plate. All data are expressed as mean ± SEM ($n = 2$, $^*P < 0.05$, $^{**}P < 0.01$, and $^{****}P < 0.001$).

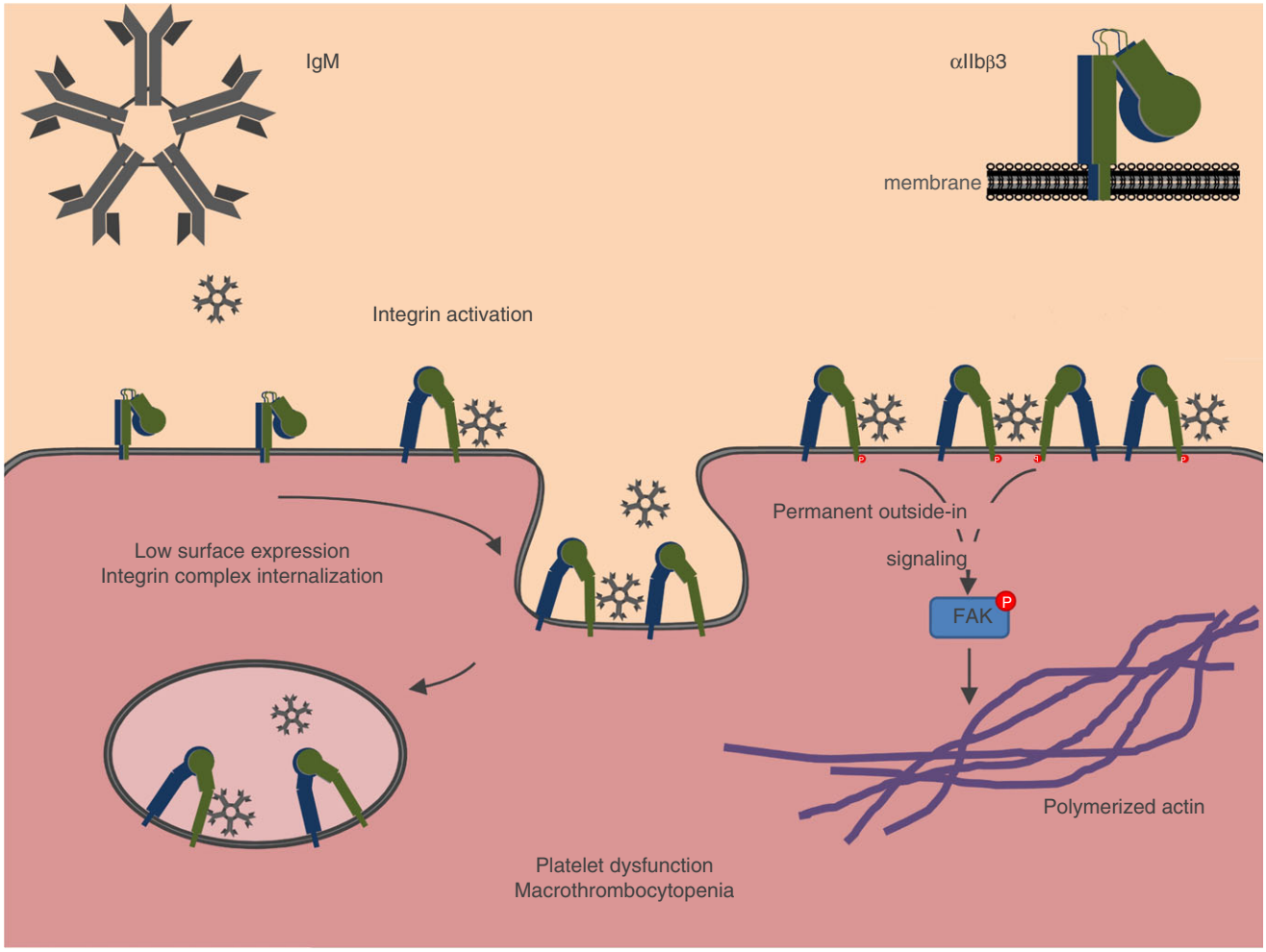


Figure 4. Mechanism by which the monoclonal IgM anti-$\alpha_{IIb}\beta_3$ autoantibody might interfere with platelet function. This autoantibody interacts with an immediate activating action leading to $\alpha_{IIb}\beta_3$ internalization which triggers outside-in signaling associated with FAK phosphorylation and cytoskeletal perturbation.

autoantibody were able to interfere with this step of megakaryopoiesis.

Management of acquired bleeding disorders bears on the treatment of the underlying cause. Here, our patient suffered from a monoclonal gammopathy of undetermined significance. Unfortunately, we did not hold data providing transition among platelet counts, $\alpha_{IIb}\beta_3$ expression, and the IgM levels. Diverse immunosuppressive drugs, such as azathioprine or rituximab, were used to treat his clonal disease, but without success. Only polyclonal intravenous immunoglobulin allowed to obtain a partial and reversible response.

In conclusion, we describe the first case of a clonal IgM causing $\alpha_{IIb}\beta_3$-mediated platelet activation and resulting in an acquired GT phenotype associated with macrothrombocytopenia. As previously described, this constitutive

activation of $\alpha_{IIb}\beta_3$ outside-in signaling is associated with altered cytoskeletal organization, platelet dysfunction, and production of giant platelets [7]. It remains to be determined whether the presence of an anti-$\alpha_{IIb}\beta_3$ activating autoantibody was responsible for an inside-out signaling dysfunction. Wencel-Drake *et al*. demonstrated, when stirring is delayed after agonist addition, that platelets lose the ability to aggregate despite the presence of maximally bound Fg, suggesting that outside-in signaling can function as an inhibitor of platelet activation [12]. More recently, Dai *et al*. showed that integrin $\alpha_{IIb}\beta_3$-mediated outside-in signals activate the Src homology 2-containing inositol 5-phosphatase-1, which mediates the disassociation of talin from β3, leading to integrin inactivation and blocking of PI3K/Akt signaling to restrict platelet secretion [13]. Thus, a constitutive and prolonged activation of $\alpha_{IIb}\beta_3$ mediated outside-in signaling could lead to a GT-like phenotype. The mechanism by which this IgM autoantibody might interfere with platelet function is summarized in Fig. 4. Although the full comprehension of the pathogenic mechanism requires further investigations, this novel clinical entity opens new perspectives for the exploration of ITP patients and regarding $\alpha_{IIb}\beta_3$ signaling in platelet production and function.

Acknowledgements

This work was supported by grants from the French Ministry of Health (DIATROC, PHRC-I 2008, API 04-07) and Novartis company (to M.F.).

Addendum

M. Fiore and X. Pillois planned the study. A. Guy realized megakaryopoiesis experiments. É. Choquet performed most of the platelet function assays. M. Fiore and J-F. Viallard followed the patient clinically. B. Payrastre and C. Garcia did western blotting for β_3 and FAK phosphorylation. J-C Bordet performed electron microscopy. M. Fiore, C. James, M. Tuffigo, and M. Jandrot-Perrus all contributed to data interpretation and writing of the manuscript.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

References

1 Coller BS. alphaIIbbeta3: structure and function. *J Thromb Haemost* 2015; **13** (Suppl. 1): S17–25.

2 Nurden AT, Pillois X, Fiore M, Alessi MC, Bonduel M, Dreyfus M, Goudemand J, Gruel Y, Benabdallah-Guerida S, Latger-Cannard V, Negrier C, Nugent D, Oiron RD, Rand ML, Sie P, Trossaert M, Alberio L, Martins N, Sirvain-Trukniewicz P, Couloux A. Expanding the mutation spectrum affecting alphaIIbbeta3 Integrin in Glanzmann Thrombasthenia: screening of the ITGA2B and ITGB3 genes in a Large International Cohort. *Hum Mutat* 2015; **36**: 548–61.

3 Kashiwagi H, Kunishima S, Kiyomizu K, Amano Y, Shimada H, Morishita M, Kanakura Y, Tomiyama Y. Demonstration of novel gain-of-function mutations of alphaIIbbeta3: association with macrothrombocytopenia and Glanzmann thrombasthenia-like phenotype. *Mol Genet Genomic Med* 2013; **1**: 77–86.

4 Nurden AT, Pillois X, Fiore M, Heilig R, Nurden P. Glanzmann thrombasthenia-like syndromes associated with macrothrombocytopenias and mutations in the genes encoding the alphaIIbbeta3 integrin. *Semin Thromb Hemost* 2011; **37**: 698–706.

5 Bury L, Malara A, Gresele P, Balduini A. Outside-in signalling generated by a constitutively activated integrin alphaIIbbeta3 impairs proplatelet formation in human megakaryocytes. *PLoS ONE* 2012; **7**: e34449.

6 Bury L, Zetterberg E, Leinoe EB, Falcinelli E, Marturano A, Manni G, Nurden AT, Gresele P. A novel variant Glanzmann thrombasthenia due to co-inheritance of a loss- and a gain-of-function mutation of ITGB3: evidence of a dominant effect of gain-of-function mutations. *Haematologica* 2018; **103**: e259–63.

7 Bury L, Falcinelli E, Chiasserini D, Springer TA, Italiano JE Jr, Gresele P. Cytoskeletal perturbation leads to platelet dysfunction and thrombocytopenia in variant forms of Glanzmann thrombasthenia. *Haematologica* 2016; **101**: 46–56.

8 Bloor AJ, Smith GA, Jaswon M, Parker NE, Ouwehand WH, Liesner R. Acquired thrombasthenia due to GPIIbIIIa platelet autoantibodies in a 4-yr-old child. *Eur J Haematol* 2006; **76**: 89–90.

9 Tuffigo M, Lazaro E, James C, Subtil C, Viallard JF, Fiore M. Successful use of recombinant factor VIIa in a patient with acquired Glanzmann thrombasthenia. *Haemophilia* 2015; **21**: e116–8.

10 Cattaneo M, Cerletti C, Harrison P, Hayward CP, Kenny D, Nugent D, Nurden P, Rao AK, Schmaier AH, Watson SP, Lussana F, Pugliano MT, Michelson AD. Recommendations for the standardization of light transmission aggregometry: a consensus of the working party from the Platelet Physiology Subcommittee of SSC/ISTH. *J Thromb Haemost* 2013; **11**: 1183–9.

11 Masse JM, Perlemuter K, Debili N, Letestu R, Castaigne A, Cramer EM. Intracellular trafficking of the alphaIIbbeta3 receptor antagonist, abciximab, in normal and Glanzmann's disease megakaryocytes. *Br J Haematol* 1999; **107**: 720–30.

12 Wencel-Drake JD, Boudignon-Proudhon C, Dieter MG, Criss AB, Parise LV. Internalization of bound fibrinogen modulates platelet aggregation. *Blood* 1996; **87**: 602–12.

13 Dai B, Wu P, Xue F, Yang R, Yu Z, Dai K, Ruan C, Liu G, Newman PJ, Gao C. Integrin-alphaIIbbeta3-mediated outside-in signalling activates a negative feedback pathway to suppress platelet activation. *Thromb Haemost* 2016; **116**: 918–30.