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Letter to the Editors-in-Chief

Multifactorial hypercoagulable state associated with a thrombotic phenotype in phosphomannomutase-2 congenital disorder of glycosylation (PMM2-CDG): Case report and brief review of the literature

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Dear Editor,

Phosphomannomutase-2 congenital disorder of glycosylation (PMM2-CDG) is a rare inherited disorder due to impaired protein *N*-glycosylation. Around 850 individuals have been recently identified in the European Reference Network EURO-CDG [1]. Only a small proportion of PMM2-CDG patients develop venous thrombosis and haemostasis exploration was most often limited to the measurement of coagulation factor and natural inhibitor activities [2–8]. The potential imbalance between prothrombotic and antithrombotic factors, especially using thrombinography, has been poorly investigated in order to explain the prothrombotic clinical phenotype.

We report here on a woman with PMM2-CDG diagnosed during childhood, characterized by compound heterozygosity of the *PMM2* gene (R141H and I153T). At the age of 28 years, she developed a spontaneous acute episode of thrombosis in the calf and popliteal veins of the left leg and received rivaroxaban during 6 months [8]. Comedications were fluticasone 250 μg/salmeterol 25 μg (spray), mometasone furoate (spray), montelukast (10 mg/day), desloratadine (5 mg/day), chlormadinone acetate (10 mg/day), estradiol (transdermal patch, 37.5 μg, twice a week), calcium carbonate (600 mg bid) and colecalciferol (100.000 IU, every three months), the last drugs being given for the treatment of severe osteoporosis. The patient had neither personal nor family history of thrombosis. As a consequence of her PMM2-CDG, she is currently able to walk only a few steps with a walking frame and mostly relies on a wheelchair. However, the chronic patient immobilization cannot be considered as a major clinical risk factor. In addition to routine parameters that we previously determined in this patient [8], we sought to thoroughly characterize coagulation phenotype including thrombinography and fibrinography, as well as other haemostasis parameters, in order to look for a potential prothrombotic phenotype.

One month after the rivaroxaban withdrawal, we confirmed on two different samples, 11-months apart, modifications in the patient's haemostatic balance, including a FXI deficiency (32 IU/dL) (normal range 60–140 IU/dL), a slight FX deficiency (65 IU/dL – N 70–130 IU/dL) and a slight increase of FVIII level (169 IU/dL - N 50–150 IU/dL) without any inflammation, associated with marked quantitative antithrombin (AT) (heparin cofactor activity 30 IU/dL - N 80–120 IU/dL) and protein C deficiencies (PC-anticoagulant activity 54 IU/dL – N 70–130 IU/dL). Patient's liver function tests were normal as well as parent coagulation parameters.

We then performed thrombinography and fibrinography (Thrombodynamics Analyser System® HemaCore, Russia) on poor platelet plasma (PPP). After triggering coagulation with tissue factor coated on an insert [9], a marked increase in thrombin generation was observed, with peak height and endogenous thrombin potential which were higher than controls, whereas temporal parameters were unchanged (Table 1, Fig. 1). In contrast to semi-global clotting times such as prothrombin time, the thrombin generation assay allows studying the coagulation system beyond clot formation, including the initiation, amplification and propagation phases, resulting in large amounts of generated thrombin over time controlled by physiological inhibitors [9]. Furthermore, fibrinography showed a markedly increased rate of clot growth whereas clot density was normal (Table 1, Fig. 1).

In addition, platelet count, closure time measured with Platelet Function Analyzer®, von Willebrand factor (VWF) ristocetin cofactor activity (VWF:RCo) were in the normal range (Table 1). To our knowledge, this is the first demonstration in PMM2-CDG of an increased proportion of intermediate and high molecular weight VWF multimers (Fig. 1). Light transmission aggregometry performed on plasma-rich platelet (PRP) showed increased platelet reactivity (slope) in the presence of ADP, arachidonic acid and collagen at different concentrations (Fig. 1). In addition, the patient's platelets displayed an increased ADP-induced activation, as shown by increased P-selectin expression measured by flow cytometry (Table 1).

One year after anticoagulant treatment discontinuation, no further thrombotic episodes have occurred. A 6-month rivaroxaban treatment had been prescribed when the thrombotic episode occurred [2]; this direct anti-Xa oral anticoagulant has the advantage of a rapid onset and requires no laboratory monitoring. Moreover, because of the AT deficiency in our patient, heparin use could have been challenging.

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Table 1
Laboratory parameters in the present patient[a].

	Patient	Reference intervals
Primary haemostasis tests		
Platelet exploration		
Platelet count (Giga/L)	368	150–450
Closure time (Platelet Function Analyser)		
• Collagen-epinephrine (s)	108	80–160
• Collagen-ADP (s)	77	59–120
Platelet aggregation tests:		
Maximal amplitude (%)/slope (%/min)		
• Spontaneous activation	< 5	< 10
• ADP 0.6 μM	**72/63**	14–25/30–50
• ADP 1.25 μM	78/80	72–86/44–96
• ADP 2.5 μM	84/**119**	76–88/64–114
• ADP 5.0 μM	80/112	79–89/74–122
• Arachidonic acid 0.8 μM	93/**197**	85–96/116–160
• Arachidonic acid 1.6 μM	96/191	87–100/120–163
• Collagen 0.4 μg/mL	LT: 32 s	LT: 52–94 s
	89/**138**	52–94/75–92
• Collagen 0.8 μg/mL	LT: 28 s	LT: 52–71 s
	89/**164**	75–92/46–124
• Collagen 1.2 μg/mL	LT: 28 s	LT 35–61 s
	88/**202**	80–115/82–146
Platelet flow cytometry activation test (%)		
• Spontaneous CD62 (P selectin)	**6**	< 5
• ADP-CD62 (P selectin)	88	60–70
• TRAP-CD62 (P selectin)	96	> 75
Von Willebrand factor (VWF)		
• VWF:RCo (IU/dL)	124	50–150
• VWF:Ag (IU/dL)	119	50–150
• VIII:C/VWFAg	1.40	> 0.60
• ADAMTS13 activity (IU/dL)	108	50–150
Fibrinography/thrombinography (Thrombodynamics Analyser System® T2-T model)		
Fibrinography		
• Rate of clot growth (μm/min)	**72.1**	34.0–44.0
• Lag time (min)	0.8	0.6–15.0
• Clot density (a.u.)	23,323	19,500–34,200
Thrombin generation assay		
• Lag time (min)	0.1	0.1–0.2
• Time to peak (min)	2.0	1.5–2.0
• Maximum thrombin concentration (AU/L)	**589**	230–410
• Endogenous thrombin potential (AU·min/L)	**6460**	1220–2170

Bold numbers indicate values out of range.
[a] Measured in the absence of anticoagulant treatment; LT: lag time; AU: arbitrary units.

The classical phenotype of PMM2-CDG includes neurological presentation, dysmorphic features and coagulopathy, with highly variable patterns [4,7]. The prevalence of venous thrombosis varies upon studies, of 2% and 7% in Schiff's and Linssen's case series, respectively, to 24% in Monin's series [5–7]. The haemostasis abnormalities include FXI, AT, and PC decreases in most patients [2–7]. This decreased AT and PC activity is not due to liver dysfunction but to other factors such as possibly an accelerated clearance of *N*-hypoglycosylated proteins. On the other hand, there is no strong association between haemostasis and clinical outcomes, possibly due to the limited number of observations and the heterogeneity of data in children and in adults [2,5–7].

Results of thrombin generation and fibrinography have not been reported yet in PMM2-CDG patients. Our results demonstrate a global hypercoagulable state in the patient despite the absence of inflammation, reflecting the disequilibrium between procoagulant and anticoagulant factors. This is associated with a markedly increased rate of clot growth on fibrinography. Whether these approaches can identify CDG patients with a high thrombotic risk needs further investigation in a large case series. Surprisingly, the patient's D-dimer levels were low (< 400 ng/mL), possibly due to altered fibrinolysis, thus deserving a comprehensive study of fibrinolysis.

Our findings of a slightly increased platelet reactivity are in agreement with the platelet hyperaggregability found in previous studies [10,11] although Arnoux et al. did not find any significant abnormality of platelet reactivity [2]. More recently, analysis of the platelet *N*-glycoproteome, including GPIbα, did not show quantitatively nor qualitatively significantly affected platelet *N*-glycoproteins in PMM2-CDG patients [11]. However, a decrease of (negatively charged) sialic acid on the platelet surface has been shown, potentially explaining platelet hyperreactivity [10,11]. Finally, we evidenced, for the first time in CDG, a high proportion of intermediate and high molecular weight multimers of VWF, without any abnormality of VWF or ADAMTS13 activity levels. Whether these abnormalities may be related to PMM2-CDG or have been found by chance in this patient cannot be stated here. VWF multimers need to be further explored in a large series of PMM2-CDG patients.

In conclusion, we confirm evidence of a hypercoagulable state associated with a high platelet reactivity and show the presence of intermediate and high molecular weight VWF multimers: all these abnormalities may have contributed to the clinical prothrombotic phenotype observed in our patient. The potential interest of the thrombin generation assay performed under our experimental

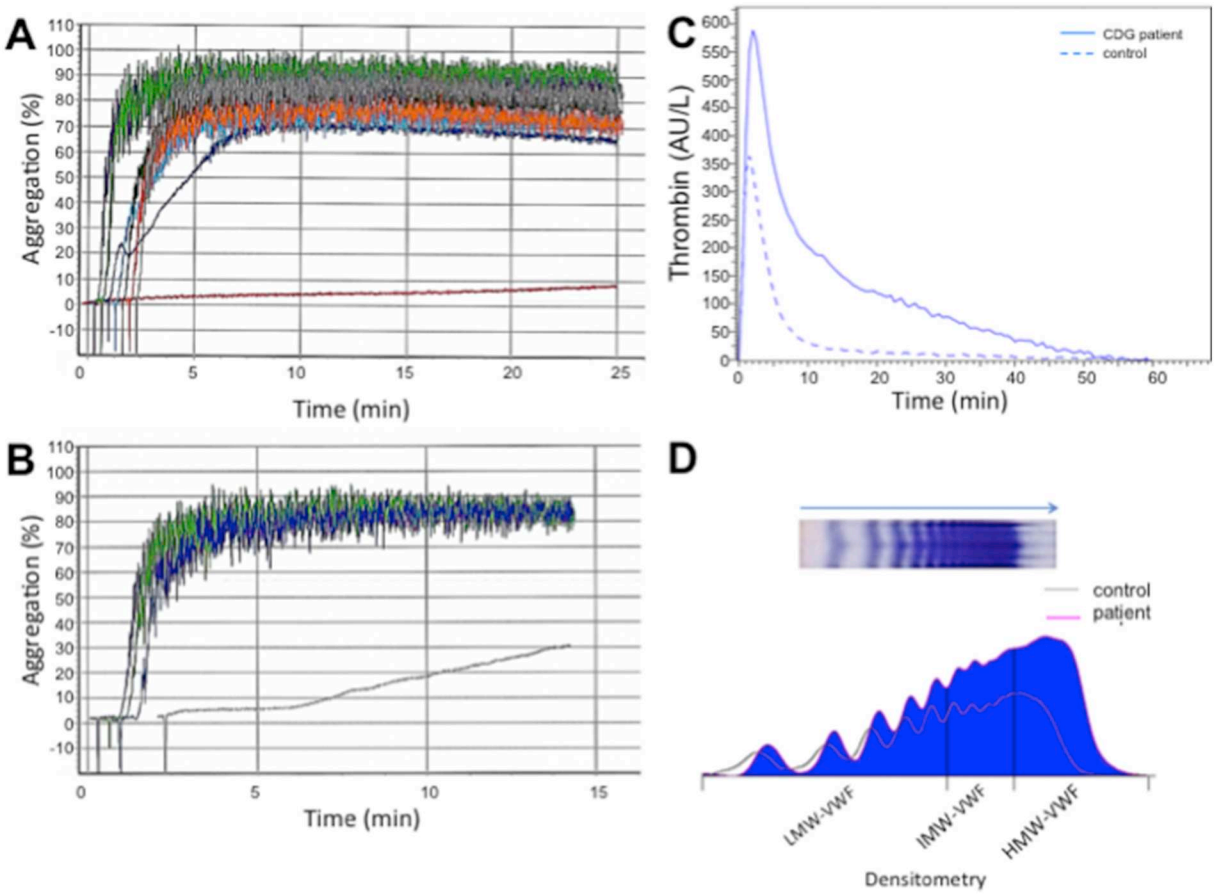

Fig. 1. Patient results of platelet light transmission aggregometry tests, thrombin generation tests and VWF multimer pattern. (A and B) platelet aggregation profiles using different agonists. (A) spontaneous (red); ADP: 0.6 µM (dark blue), 1.25 µM (pale blue), 2.5 µM (black), 5 µM (orange), 10 µM (grey); arachidonic acid: 0.25 mg/L (purple), 0.5 mg/mL (green); (B) collagen: 0.4 µg/mL (pale purple), 0.8 µg/mL (green), 1.2 µg/mL (blue); (C) Thrombin generation profile (Thrombodynamics analyser System® T2-T model); (D) VWF multimer pattern after electrophoresis (Hydrasis2®). LMW: Low Molecular Weight multimers, IMW Intermediate Molecular Weight multimers, HMV: High Molecular Weight multimers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conditions and other tests such as fibrinography remain to be confirmed in a series of PMM2-CDG patients, in order to possibly identify patients at particular risk of thrombosis. Finally, in patients in whom the use of anticoagulants is challenging due to frequent AT deficiency, rivaroxaban can be proposed as an attractive therapeutic option in the treatment of venous thromboembolism.

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Author contributions

P.C. and L.D. managed the patient, B.L., A.S., V.S., G.F.P, N.B., and M.A.G. performed laboratory analysis and analyzed data, B.L. and V.S. wrote the manuscript and all authors critically discussed data and gave final approval.

Conflicts of interest

None.

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