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Coagulation abnormalities in a prospective cohort of 50 patients with PMM2-congenital disorder of glycosylation

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Abstract

Background: Given the lack of reliable data on the prevalence of bleeding abnormalities and thrombotic episodes in PMM2-CDG patients, and whether coagulation abnormalities change over time, we prospectively collected and reviewed natural history data. Patients with PMM2-CDG

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Authors contributions

DDG, JR and EM were involved in conceptualization, designed experiments, and wrote the manuscript. DDG, JR, AL and CJ were responsible for data collection. DDG, JR, GLM, AL, GP, CL, ACE, TK, and EM were involved in the data evaluation. All authors were involved in the interpretation of data and reviewing and editing the manuscript.

Ethics approval

All patients included in this work are enrolled in the Frontier in CDG Consortium (FCDGC) natural history study (institutional review board (IRB) 19–005187; https://clinicaltrials.gov/ct2/show/NCT04199000?cond=CDG&draw=2&rank=4). Written informed consent was obtained from the legally authorized representatives of the subjects prior to study initiation.

Conflict of interest statement

Diederik De Graef, Anna N. Ligezka, Joseph Rezents, Gina L. Mazza, Graeme Preston, Kaitlin Schwartz, Wirginia Krzysciak, Christina Lam, Andrew C. Edmondson, Christin Johnsen, Tamas Kozicz, and Eva Morava report no conflict of interest.

often have abnormal coagulation studies due to glycosylation abnormalities but the frequency of complications resulting from these has not been prospectively studied.

Methods: We studied fifty individuals enrolled in the Frontiers in Congenital Disorders of Glycosylation Consortium (FCDGC) natural history study with molecularly confirmed diagnosis of PMM2-CDG. We collected data on prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), platelets, factor IX activity (FIX), factor XI activity (FXI), protein C activity (PC), protein S activity (PS) and antithrombin activity (AT).

Results: Prothrombotic and antithrombotic factor activities were frequently abnormal in PMM2-CDG patients, including AT, PC, PT, INR, and FXI. AT deficiency was the most common abnormality in 83.3% of patients. AT activity was below 50% in 62.5% of all patients (normal range 80–130%). Interestingly, 16% of the cohort experienced symptoms of spontaneous bleeding and 10% had thrombosis. Stroke-like episodes (SLE) were reported in 18% of patients in our cohort. Based on the linear growth models, on average, patients did not show significant change in AT (n=48; t(23.8)=1.75, p=0.09), FIX (n=36; t(61)=1.60, p=0.12), FXI (n=39; t(22.8)=1.88, p=0.07), PS (n=25; t(28.8)=1.08, p=0.29), PC (n=38; t(68)=1.61, p=0.11), INR (n=44; t(184)=−1.06, p=0.29), or PT (n=43; t(192)=−0.69, p=0.49) over time. AT activity positively correlated with FIX activity. PS activity was significantly lower in males.

Conclusion: Based on our natural history data and previous literature, we conclude that caution should be exercised when the AT levels are lower than 65%, as most thrombotic events occur in patients with AT below this level. All five, male PMM2-CDG patients in our cohort who developed thrombosis had abnormal AT levels, ranging between 19% and 63%. Thrombosis was associated with infection in all cases. We did not find significant change in AT levels over time. Several PMM2-CDG patients had an increased bleeding tendency. More long-term follow-up is necessary on coagulation abnormalities and the associated clinical symptoms to provide guidelines for therapy, patient management, and appropriate counseling.

Keywords

CDG; glycosylation; factor XI; antithrombin; thrombosis; abnormal coagulation; bleeding; thrombosis

INTRODUCTION

In humans, glycosylation starts in the cytoplasm and continues in the endoplasmic reticulum and the Golgi apparatus. Proper glycosylation is vital for producing properly functioning glycoproteins, glycolipids and glycosylphosphatidylinositol anchored proteins[1]. When glycosylation fails, the functionality of the proteins is greatly reduced or even lost, causing severe abnormalities in development and metabolism[1]. Additionally, aberrantly glycosylated proteins have higher turnover rates due to endocytosis by the liver, lowering their levels in the bloodstream.[2] Congenital Disorders of Glycosylation (CDG) are a heterogeneous group of inherited metabolic disorders caused by a defect in various steps along the glycosylation pathways. There are some 170 CDG known at the time of publication, and they vary in severity as well as in incidence rate[3–5]. Phosphomannomutase 2-congenital disorder of glycosylation (PMM2-CDG, MIM #212065) is the most common N-glycosylation disorder, with more than 900 patients worldwide reported in the literature so far[6]. PMM2-CDG affects

almost all organs and organ systems, leading to neurologic, ophthalmologic, gastrointestinal, cardiac, skeletal, and endocrine symptoms[7].

Importantly, laboratory analysis of coagulation is frequently abnormal in PMM2-CDG patients[8]. According to the international clinical guidelines for PMM2-CDG, abnormal coagulation has been reported in more than 300 patients in the literature[9]. These often include abnormal procoagulant factors and anticoagulant factors, even in the same patient. Coagulation factors IX (FIX) and XI (FXI) are important markers of PMM2-CDG, along with antithrombin (AT), protein C (PC), and protein S (PS)[10]. Both abundance and activity of these factors are commonly measured as low (both for abundance and for activity) in PMM2-CDG patients and can pose a potential risk for bleeding and thrombotic complications[10]. However, there is limited research around the clinical relevance of these coagulation defects, the actual risk for bleeding diathesis and thrombotic events in PMM2-CDG patients, and the significance of the reduced coagulation factors in these patients.

Current literature on the prevalence and management of coagulation abnormalities in PMM2-CDG patients is limited to retrospective studies, clinical expertise, and case reports. Here, we performed the first study on prospectively collected data, in addition to retrospective data, to investigate the relevance of coagulation abnormalities associated with PMM2-CDG, to improve our knowledge on the incidence of complications related to laboratory abnormalities, and if these change over time.

METHODS

Subjects

Patients with a molecularly confirmed diagnosis of PMM2-CDG were included in the study. All patients are enrolled in the Frontiers in Congenital Disorders of Glycosylation Consortium (FCDGC) natural history study upon written and informed consent (Institutional Review Board [IRB]:19–005187; https://clinicaltrials.gov/ct2/show/NCT04199000?cond=CDG&draw=2&rank=4). Data was collected prospectively, and retrospective data was available for many patients as well, if patients have been previously followed-up by standard of care. The mean follow-up time for the prospective study was 20.2 months and median follow-up time was 20.5 months. The shortest follow up time was 5 months, as patient 33 (P33) was enrolled at 1 month old and died at age 6 months. The longest follow up time was 38 months (P37).

Data extraction

Data was collected and extracted from the electronic clinical files and electronic laboratory data system. The following laboratory studies on proteins regulating coagulation were collected as part of the natural history study according to clinical care: prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), platelets, factor IX activity (FIX), factor XI activity (FXI), protein C activity (PC), protein S activity (PS) and antithrombin activity (AT). All studied coagulation factors are glycosylated proteins and collected according to clinical guidelines[9,11–17].

Mono-oligo/di-oligo ratio and A-oligo/Di-oligo ratio from mass spectrometry of glycosylated transferrin testing were collected for each patient. Carbohydrate deficient transferrin (CDT) testing measures serum transferrin N-glycosylation and is the gold standard test for screening, and for assessing therapeutic outcomes[18].

Clinical vascular events including both bleeding and thrombotic events were extracted from the electronic clinical files by a systematic search of the following keywords: “bleeding”, “bruising”, “surgery”, “clotting”, “thrombosis”, “DVT”, “embolism”, “stroke”, “vascular” and “hematology”. Available data was selected on clinical relevancy. We also reviwed the use of thrombosis prophylaxis and in female patients, estrogen replacement therapy and concurrent thrombosis prophylaxis.

The Nijmegen Patient CDG Rating Scale (NPCRS) was performed in every patient at the time of the laboratory sample collection. The NCPRS is a clinical tool developed to determine the clinical severity and disease progression of patients with CDG in an objective way and is validated for all age groups. Depending on the NPCRS score, patients are scaled in a mild (0–14), moderate (15–25) and severe (>26) category.[19]

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 8.3 (Dotmatics, USA) or SAS 9.4 (SAS Institute Inc., Cary, NC). For categorical variables, frequencies and percentages are provided; for continuous variables, descriptive statistics, including sample size, mean, median, standard deviation, and range of values (i.e., minimum and maximum values), are provided. Patients’ mean laboratory values across time were averaged to obtain the mean laboratory value for the full sample. Patients with at least one measurement of a given coagulation factor were included in all plots and analyses for that coagulation factor. As an exploratory analysis, we estimated the within-patient and between-patient correlation between AT activity and Factor XI activity.

For each coagulation factor, we estimated a linear growth model with a random intercept and random slope for time since first measurement (in months). Time since each patient’s first measurement was selected as the time metric of interest so that the linear growth model parameters would reflect within-patient change over time without consideration for age. If we encountered convergence issues due to the random slope variance being close to zero, we estimated the linear growth model without a random slope for time since first measurement. As a supplemental analysis, sex differences in the coagulation factors were evaluated by including sex in the linear growth models. Statistical significance was defined as $p<0.05$ for all analyses.

In the subsample of patients with two or more measurements of AT activity, we calculated percent change between each patient’s first and last measurement (i.e., [last measurement – first measurement] / first measurement × 100%). To examine normalization of patients’ AT activity over time, we summarized the number of patients classified as abnormal at their first measurement who were classified as normal (i.e., AT activity of 80% to 130%) at their last measurement.

RESULTS

Patients

Fifty patients, with ages ranging from 6 months to 35 years, were included in the study. All patients were clinically followed at our CDG outpatient clinic at Mayo Clinic, Rochester. The median age was 8 years (range: 6 months to 35 years) and 66% (33/50) of participants were male. Seven patients were older than 18 years, 57% of them were female (4/7). Detailed patient characteristics can be found in Table 1.

Molecular findings

All 50 patients had genetically confirmed PMM2-CDG diagnosis. The most common variant was c.422G>A (p.Arg141His), 33/100 (33%) PMM2 alleles consisted of this variant in our 50 patients. We found only one homozygous variant: c.357C>A (p.Phe119Leu). Genotypes of all patients are displayed in Table S1.

Laboratory findings: coagulation studies

Main findings for coagulation markers in the overall cohort and stratified for children, adults, female, and male patients are shown in Table 1. Laboratory findings for all patients individually are shown in Table 2 and Figure S1. We found that PMM2-CDG affected the activity of anticoagulant and procoagulant factors, as well as clotting time and platelet levels. Levels of AT, PC, and PS were abnormal in 83.3% (40/48), 73.7% (28/38), and 42.3% (11/26) of patients respectively. FXI activity was low in 51.3% (20/39), whereas FIX activity was low in only 27.8% of patients (10/36). PT was high in 62.7% (27/42) of patients and INR was high in 46.5% (20/43) of patients. Of the 42 patients with available aPTT values, 13 (31.0%) had abnormal results. Platelet levels were available for 41 patients: of these, 18 (43.9%) had abnormal values. The mean value was 312.4 (normal 187–400 K/μL). Platelet levels were decreased in 9 patients, fluctuated between both increased and decreased in 2 patients, and 7 patients had elevated levels.

Interestingly, AT activity and Factor XI activity were strongly positively correlated within patients (r_{within}=0.72) and between patients ($r_{between}$=0.88).

Total protein levels were available for 40 patients: 20 patients (50.0%) had abnormal levels (normal 6.3–7.9 g/dL). 18 patients had decreased levels, one patient had increased levels, and one patient had both decreased and increased levels.

Based on the linear growth models, on average, patients did not show significant change in AT activity (n=48; t(23.8)=1.75, p=0.09), FIX activity (n=36; t(61)=1.60, p=0.12), FXI activity (n=39; t(22.8)=1.88, p=0.07), PS activity (n=25; t(28.8)=1.08, p=0.29), PC activity (n=38; t(68)=1.61, p=0.11), INR (n=44; t(184)=−1.06, p=0.29), or PT (n=43; t(192)=−0.69, p=0.49) over time. Figure 1 shows patients' predicted linear growth curves for AT activity and FXI. For AT activity, 40 patients had two or more measurements, with the median time between the first and last measurement being 26.8 months (range=1.3 to 109.8 months). The percent change between each patient's first and last measurement was highly variable (median=13.6%, range=−46.6% to 203.0%). All 8 patients with normal AT activity at their

first measurement (i.e., AT activity of 80% to 130%) had normal AT activity at their last measurement. Of the 32 patients with abnormal AT activity at their first measurement, 26 (81.3%) had abnormal AT activity at their last measurement.

Sex did not significantly predict AT activity (n=48; t(46.4)=1.77, p=0.08), FIX activity (n=36; t(33.6)=1.22, p=0.23), FXI activity (n=39; t(37.8)=1.66, p=0.11), PC activity (n=38; t(36.1)=1.76, p=0.09), INR (n=44; t(22.4)=0.01, p=0.99), or PT (n=43; t(22.7)=−0.56, p=0.58). On average, PS activity was 22.78 points lower in male patients than in female patients (n=25; t(22.8)=3.17, p=0.004).

Clinical findings

Clinical findings available for all 50 patients, followed at our outpatient clinic, are summarized in Table 3. Prolonged or excessive bleeding or bruising was the most frequent vascular event (AE) in our cohort: 8 patients (16%) reported having frequent bleeding and/or bruising. Of note, one patient (P17) presented with bleeding originating from esophageal varices. Four patients (8%) reported having frequent epistaxis, presenting with nose bleeds between the age of 11 months to 11 years. Hematemesis and hematochezia were reported in 5 patients (10%). Three patients reported hematochezia, one presented with hematemesis, and one suffered from both hematemesis and hematochezia. Both cases of hematemesis were isolated episodes at 2 years old and 10 years old in P28 and P48 respectively. Hematochezia was recurrent in P20 and P38 but was an isolated event in P28 and P36. Perioperative bleeding was reported in 4 patients (8%)—two patients during infancy (9 months old and 11 months old respectively) and two in adolescence (15 years old). In all cases transfusion of Packed Red Blood Cells (PRBC) and Fresh Frozen Plasma (FFP) was necessary peri- or postoperatively. While patient 36 received FFP preoperatively, bleeding still occurred during surgery and required additional treatment with FFP and PRBC.

Five patients (10%) suffered a deep venous thrombosis (DVT). P1 developed multiple DVTs of the left brachial vein at the age of 3 years. At the time of the event, he was suffering from an infection and was dehydrated, and his AT level was low at 63%. During a septic episode, P12 experienced coagulopathy with reported AT levels around 40%. This resulted in a DVT in the left popliteal vein at age 9 months. One patient (P31) acquired a first DVT in the left lower extremity at 20 years old. Unfortunately, at the age of 29 years, P31 was hospitalized due to COVID-19 infection and a pneumothorax. During this period of immobilization, he suffered another DVT in the left lower extremity. On both occurrences, P31 had very low AT levels of 21% and 32%, respectively. P33 developed a portal venous thrombosis at the age of 8 months old. His AT value at the time of thrombosis was 46%. One patient developed a DVT in the lower extremities during the neonatal period at 11 days old (P40). He was treated in the neonatal intensive care unit (NICU) with antibiotics for a possible sepsis at the time of thrombosis. AT levels at that time are not recorded. However, upon enrollment in the study at age 8 months he had an AT level of 44%.

Nine patients (18%) in our cohort had a history of stroke-like episodes (SLE). All of them had episodes of transient hemiparesis lasting for 24 hours or longer, without imaging signs of stroke on MRI or CT, and without seizure like activity on EEG. Two of the patients had somnolence and transient encephalopathy. No thrombotic episodes were confirmed, and

the motor function spontaneously recovered in all individuals. Patients who had seizures prior to the SLE were treated for their seizures both acutely and long term, and none of the patients were known with migraines. Four patients (P8, P10, P33 and P42) developed a SLE in the context of a febrile illness. A period of high fever and persistent vomiting was followed by SLE in P17. One patient (P44) suffered a SLE after a head trauma. SLE with left hemiparesis presented in P27 after a prolonged seizure. P36 had multiple SLEs starting at the age of 17 years. His episodes occurred either after head trauma or during a period of fever followed by vomiting and dehydration. In one patient (P48) the SLE was associated with hypoglycemia. P32 and P33, twin brothers with identical genotypes, unfortunately died at respectively 6 months and 12 months old. P33 suffered of liver failure and a portal venous thrombosis, as mentioned above.

Treatments

After acquiring a DVT, P31 and P40 were prescribed direct oral anticoagulants (DOACs) to prevent thrombosis. P31 is treated with edoxaban indefinitely while P40 was treated with rivaroxaban for 3.5 months. Three patients (P1, P31 and P40) have been treated with heparin (e.g. enoxaparin). P38 and P46 take a low dose of aspirin to prevent DVTs and thrombosis from occurring. Two patients in our cohort, P4 and P5, have been prescribed aminocaproic acid as needed in the case of excessive bleeding. Six patients (P1, P12, P17, P24, P36 and P43) have received PRBC and/or FFP perioperatively.

All four female patients above the age of 16 years (P18, P19, P38 and P48) have been prescribed estrogen therapy (estradiol patch or oral) to induce puberty and mediate symptoms due to hypergonadotropic hypogonadism. P38 has been prescribed aspirin as thrombosis prophylaxis. (Patient characteristics displayed in Table S2.)

Nijmegen Patient CDG Rating Scale (NPCRS)

The NPCRS score of all 50 patients was collected. The median total score was 22 (moderate severity), ranging from 9 to 37. The median scores of Section I, Section II and Section III were respectively 8, 3 and 11. The NPCRS scores of all patients are shown in Table S3.

Patients with clinical findings regarding coagulation typically had a moderate or severe NPCRS score. Three patients reporting easy bleeding/bruising had a moderate score, five had a severe score. Epistaxis presented in one mild, one moderate and two severe patients, while excessive bleeding during surgery occurred in two moderate and two severe cases. Hematemesis or hematochezia was found in one mild, three moderate and one severe patient. Two moderate and three severe patients suffered from a DVT. One mild, three moderate and five severe patients reported SLE. (NPCRS scores for each symptomatic patient included in Table 3.)

DISCUSSION

In this prospective study, we found that prothrombotic and antithrombotic proteins are abnormal in more than 80% of PMM2-CDG patients. We confirmed that the most frequently abnormal pro- and anticoagulant parameters are AT, PC, PT, INR, and FXI. AT deficiency was the most common coagulation abnormality in PMM2-CDG according to our results,

comparable with previous retrospective findings. In our cohort AT was abnormal in 83.3% of patients (40/48). According to a previously published study, one in three patients had AT activity levels below 50%[20]. However, our study showed that AT activity levels were below 50% (normal range 80–130%) in 62.5% of patients at least once during the evaluations (30/48). The INR and PT showed in 46.5–62.8% of patients increased values. FIX, PS, aPTT and platelets were also documented to be abnormal in several patients. In our natural history data, we observed transient decrease in intraindividual factor levels during episodes of infections or stress in four patients (P27, P29, P39, and P41). Based on the linear growth models, on average, the patient cohort did not show significant change in AT activity or Factor XI activity over time. We concluded, that as fluctuations can occur, annual evaluation of coagulation factors should be recommended for all patients.

Bleeding episodes or easy bruising was the most frequently reported complication of coagulopathy in our cohort. Perioperative bleeding, epistaxis, hematemesis, and hematochezia were also reported as bleeding complications. In all cases of perioperative bleeding a blood transfusion was necessary. Ten percent (5 out of 50) of PMM2-CDG patients in our cohort developed a DVT, in association with an infection. All four patients with available lab results had low AT activity levels at the time of DVT. (Unfortunately for P40 we do not have an AT level at the time of event.) Similarly, a previous cohort study by Linssen et al. described 14 out of 100 PMM2-CDG patients developed a DVT and 10 of them had significantly decreased AT levels.[8] Thrombosis occurred predominantly in young children, and none of them developed recurrence[21]. One patient had recurrent DVTs and developed them in adulthood. Infection, especially in combination with dehydration and immobilization, might be an indication for thrombosis prophylaxis with anticoagulant therapy in young PMM2-CDG patients.

A recent publication described the occurrence of DVT in adolescent female CDG patients on estrogen replacement with a history of delayed puberty.[22] In our cohort, all four female patients above the age of 16 years have been placed on estrogen therapy and as coagulation factors have been normal in most cases, only one, with abnormal levels has been prescribed prophylactic anticoagulation (See Table S2). Based on the fluctuating AT levels detected in our study in some of the patients, and the recent report, estrogen replacement therapy may warrant prophylactic anticoagulation, either by antiplatelet therapy (e.g. aspirin) or DOACs (e.g. rivaroxaban).

Interestingly, all patients with a history of DVT were male. Epistaxis and perioperative bleeding were also reported exclusively in males. Mean levels of endogenous anticoagulant factors (i.e., AT, PC, and PS) were lower in male patients but the difference only reached statistical significance for PS activity (See Table 1). All other coagulation factors did not significantly differ by sex. These observations may indicate that male PMM2-CDG patients, especially those with low PS activity, are particularly at risk for thrombosis. It is worth noting that we expected a higher risk in females, especially on estrogen therapy[22]. Based on the data one should suggest to regularly follow Protein S activity in males.

Stroke-like episodes (SLE) are known to be prevalent in PMM2-CDG but are not clearly related to coagulation abnormalities.[8] SLE were frequently reported in our cohort (18%)

and were diagnosed based on clinical symptoms, absence of stroke on MRI or CT, and no seizure like activity on EEG. The exact cause of SLE in CDG has not yet been identified.[9] Multiple hypotheses have been formulated such as a vascular origin and/or metabolic origin.[23–25] Cerebrovascular factors may be partly responsible for the development of SLE.[26] The cause of vasculogenic SLE could be related to the acute, and transient imbalance in anticoagulant proteins (i.e., AT, PC, and PS). pInterestingly, all patients with a history of stroke-like episodes and/or thrombotic events had hypercoagulant TGA results.[29] We found that AT activity and FXI activity are also strongly positively correlated in our cohort. The literature suggests that low clotting factors could have a preventative effect when decreased in parallel with AT deficiency. The low number of thrombotic episodes in our group of CDG patients compared to primary AT deficiency could be due to the counterbalance of low FXI, and potentially also other procoagulant factors. However, patients in our own cohort who developed DVT had both low AT and low clotting factor levels. The discrepancy could be explained by additional stress factors such as infection or fever.

[25] In a large retrospective multicentric cohort study of 968 patients with primary AT deficiency, de la Moreno-Barrio et al. found 7.5% of patients developed a thrombotic event. Of the patients with a thrombotic event and an available AT activity level, 83.7% (36/43) had an AT activity level of 65 or less (Normal range: 80–130%)[27]. We cannot conclude on an exact cut off level for the risk for thrombosis in PMM2-CDG. Based on our natural history data and previous literature, we conclude that caution should be exercised when the AT activity levels are lower than 65% (reference range: 80–130), as the majority of thrombotic events occur in patients with AT below this level. Disequilibrium between pro-coagulant and anti-coagulant factors is believed to be the underlying pathological mechanism for bleeding and thrombotic events.[8,28] A study by Pascreau et al.[29], provides a basis to further interpret the coagulation abnormalities seen in CDG patients. Similar to our data and what is previously described in the literature, they found that AT and FXI are the main coagulation factors affected, followed by PC, PS and FIX respectively. The study used Thrombin Generation Assay (TGA) to assess the impact of combined deficiencies of procoagulant and anticoagulant factors on the hemostatic balance in patients with CDG. Their results evidenced a hypercoagulant state in vitro, likely due to lower AT activity and impairment of the PC system. However, the researchers hypothesized that in vivo the procoagulant effect of AT deficiency and PC system impairment is counterbalanced by the lower level of FXI. Their analysis showed a strong correlation between AT and FXI levels in the study population.

We propose that clinical management should consist of yearly follow-up of bleeding and clotting parameters (PT, PC, PS, AT, FIX, and FXI) and parent education for warning signs[10]. Coagulation studies should be repeated once a year and before surgery, invasive procedure or during illness or environmental stress.[9] FFP should be administered to patients during surgery who have an increased bleeding tendency. After a patient develops a DVT, low molecular weight heparin treatment (LMWH) is often prescribed to prevent recurrent DVT in the following 6 weeks to 6 months, depending on severity and the presence of provoking factors.

Data on the efficacy of antithrombotic treatment in PMM2-CDG is limited. There has been a report of a patient with primary AT deficiency where LMWH treatment was inefficient because heparins must bind to AT to function. Given the prevalence of AT deficiency in PMM2-CDG, there might be a concern that LMWH has lower efficacy in CDG patients as well.[9] However, it has also been described that hypoglycosylated isoforms of antithrombin, missing the N-glycan chain at Asn135, have greater affinity for heparin.[29–31] While patients' AT levels are often reduced, this could be counteracted by the improved affinity of the residual AT to heparin. As mentioned in the clinical guidelines, DOACs (e.g., rivaroxaban) have been successfully used as alternative prophylaxis in PMM2-CDG.[9] In our cohort, two patients who remained at risk of thrombosis were given DOACs to prevent excessive clotting. In case a procedure is necessary, a hematologist should always be consulted on the proper course of action regarding the anticoagulant therapy.

Coagulation studies can be used to confirm diagnosis of PMM2-CDG as the abnormalities in both procoagulant and anticoagulant factors is pathognomonic for CDG. Next to their clinical relevance, AT and FXI are excellent biomarkers for CDG and could be potential biomarkers for future clinical trials. Although a few patients normalized their coagulation data, based on the linear growth models, on average, patients did not show significant change in the activity of AT, FIX, FXI, PS, PC, or in PT over time. . In future clinical trials, especially those aimed at improving glycosylation, like the one currently underway (NCT04925960), this observation is crucial.

CONCLUSION

We conclude that yearly follow up for PT, PC, PS, AT, FIX, and FXI activities, as directed by the current JIMD guidelines, is essential for appropriate clinical care in PMM2-CDG. As thrombotic episodes are life-threatening, patients with AT deficiency should be aware of clinical warning signs, and parents should be educated for the necessary emergency strategy. As most PMM2-CDG patients never develop thrombotic episodes, preventive anticoagulants should be only recommended in patients with a positive thrombotic history or patients receiving estrogen replacement therapy. In addition, AT and FXI activity are reliable potential biomarkers for future clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CDG	Congenital disorders of glycosylation

PMM2	Phosphomannomutase 2
AT	Antithrombin
PT	Prothrombin time
INR	International normalized ratio
aPTT	Activated partial thromboplastin timea
CDT	Carbohydrate deficient transferrin
FVIII	Coagulation factor VIII
FIX	Coagulation factor IX
FXI	Coagulation factor XI
PC	Protein C
PS	Protein S
SLE	Stroke-like episode
NCPRS	Nijmegen CDG Patient Rating Scale
DVT	Deep venous thrombosis
DOAC	Direct oral anticoagulants
PRBC	Packed Red Blood Cells
FFP	Fresh frozen plasma
LMWH	Low molecular weight heparin

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Synopsis:

Most PMM2-CDG patients display chronic coagulation abnormalities without significant improvement, associated with a frequency of 16% clinical bleeding abnormalities, and 10% thrombotic episodes in patients with severe antithrombin deficiency.

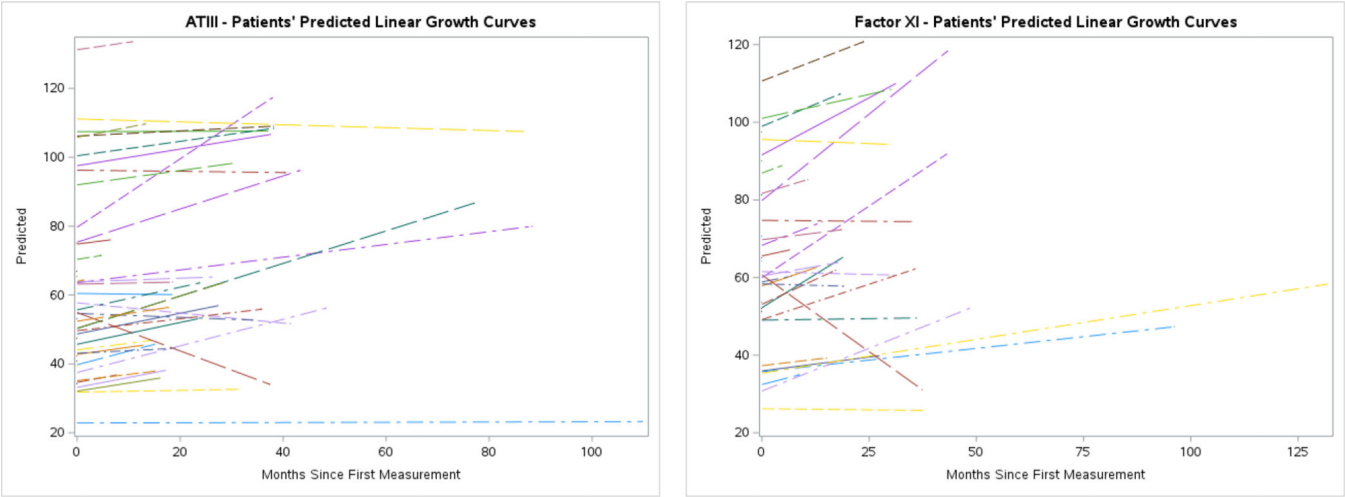

Figure 1.

Patients' predicted linear growth curves for AT activity (left) and Factor XI (right). Based on the linear growth models, on average, patients did not show significant change in AT activity (n=48; t(23.8)=1.75, p=0.09) or Factor XI activity (n=39; t(22.8)=1.88, p=0.07). The linear growth model for AT activity included a random slope for time since first measurement (in months), whereas the linear growth model for Factor XI did not. The normal range is 80% to 130% for AT activity and 55 to 150 for Factor XI activity.

Table 1.

Patient characteristics (n=50) and main findings for coagulation markers and NPCRS scores in the overall cohort and stratified for children, adults, female, and male patients.

Patient characteristics (n=50)	**no. (%)**						
Age group							
0–1 years	2 (4)						
2–11 years	35 (70)						
12–20 years	7 (14)						
>20 years	6 (12)						
Sex							
Male	33 (66)						
Female	17 (34)						
Coagulation markers (normal range)	**Mean (SD)**	**Number of datapoints**	**Abnormal values (%)**	**Children (0-18 years) Mean (SD)**	**Adults (>18 years) Mean (SD)**	**Female Mean (SD)**	**Male Mean (SD)**
Antithrombin (80–130%)	62.9 (28.1)	214	40/48 (83.3)	62.9 (26.8)	62.6 (39.1)	73.4 (29.8)	57.6 (26.2)
INR (0.9–1.1)	1.10 (0.16)	202	20/43 (46.5)	1.11 (0.17)	1.07 (0.09)	1.09 (0.09)	1.11 (0.18)
PT (9.4–12.5 sec)	12.8 (2.00)	194	27/43 (62.8)	12.9 (2.12)	12.4 (0.935)	12.5 (1.39)	12.9 (2.27)
aPTT (25–37 sec)	32.9 (5.27)	138	13/42 (31.0)	33.1 (5.58)	31.4 (2.67)	32.5 (4.13)	33.0 (5.83)
Protein C activity (70–150%)	65.3 (27.7)	99	28/38 (73.7)	63.2 (23.2)	76.7 (46.4)	75.1 (33.5)	59.6 (22.5)
Protein S activity (60–140%)	71.0 (19.6)	53	11/26 (42.3)	69.9 (17.3)	75.7 (29.3)	84.5 (20.8)	62.7 (13.6)
Factor IX activity (65–140%)	80.3 (17.8)	85	10/36 (27.8)	76.8 (17.0)	98.1 (9.32)	85.0 (17.9)	77.7 (17.6)
Factor XI activity (55–150%)	67.0 (24.2)	144	20/39 (51.3)	67.4 (23.7)	64.5 (29.2)	73.8 (25.2)	63.2 (23.3)
Platelets (187–400 ×109/L)	312 (106)	202	18/41 (43.9)	321 (111)	261 (40.3)	321 (134)	308 (90.9)
The Nijmegen Patient CDG Rating Scale, NPCRS [range]	**Mean (SD)**			**Children and adolescents (0–18 years) Mean (SD)**	**Adults (>18 years) Mean (SD)**	**Female Mean (SD)**	**Male Mean (SD)**
Section I: Current Function [0–21]	8 (3.2)			8 (3.2)	7 (3.2)	8 (3.18)	8 (3.15)
Section II: System Specific Involvement [0–30]	3 (2.5)			4 (2.6)	3 (1.5)	3 (1.68)	3 (2.84)
Section III: Current Clinical Assessment [0–27]	11 (2.8)			11 (2.4)	10 (4.7)	10 (2.94)	11 (2.60)
Total score: [0–78]	22 (6.2)			23 (5.8)	20 (8.1)	20 (6.09)	23 (6.15)

Table 2.

Range of laboratory values for each patient.

Patient (#)	Sex (M/F)	Age (Y)	Platelets (x10^9/L)	INR (ratio)	aPTT(sec)	PT (sec)	Factor IX (%)	Factor XI (%)	Protein C (%)	Protein S (%)	Antithrombin (%)	Genotype	Events
Reference values:			*187–400*	*0.9–1.1*	*25–37*	*9.4–12.5*	*65–140*	*55–150*	*70–150*	*60–140*	*80–130*		
1	M	3	**12–361**	**0.9–1.3**	**41**	**10.7-15.8**	**55–66**	**32–39**	**46–82**	66–90	**34–63**	p.Gly15Ala; p.Arg141His	Perioperative bleeding, DVT
2	M	10	254-284	**1.1–1.3**	32-33	**12.0–13.8**	83–84	66–67	**60–81**		**74–80**	p.Ala108Val; p.Thr237Arg	
3	M	3	**175–354**	**0.9–1.2**	25	**10.5–13.7**	**56–65**	**40–88**	**55**	**47**	**28–71**	p.Arg141His; p.Phe68Cys	
4	F	11	**113–151**	1.0		**13.0**	105	**48**	77	114	**68**	p.Arg141His; p.Glu208Ala	Easy bleeding and bruising
5	F	8	**181–348**	1.0-1.1	29–33	11.0–12.4	82–99	78–118	94–100		87–119	p.Arg141His; p.Glu139Lys	
6	F	2		**1.2–1.3**	**35–41**	**15.8–16.7**					**21–37**	p.Arg141His; p.Asn216Ile	
7	M	12	**177–320**	1.0	28		75	72		68–81	**48–72**	p.Arg141His; p.Pro113Leu	
8	M	7							**42**	**46**	**38**	p.Pro113Leu; pVal182Asp	SLE
9	F	6	375–379	**1.1–1.3**	21–32	**11.6–14.3**	74	**51–68**	**56**		**40–44**	p.Arg141His; p.Val129Met	
10	F	8	249–297	1.0–1.1	25–35	11.0–12.0	**63–78**	96	**69–81**	80–101	103–119	p.Thr237Arg; p.Cys241Ser	Easy bleeding and bruising, SLE
11	F	5	335–390	1.0	33	10.3–12.4	**57–81**	64	**23–51**	**33–69**	**24–41**	p.Arg141His; p.Asn216I1e	
12	M	3	308–605	**1.4**	28	**17.5**	**62**		**20**	**58**	**16–34**	p.Arg141His; p.Asn216Ile	Perioperative bleeding, DVT
13	M	3	351	1.1	29	12.1		65	**69**		**51**	p.Arg141His; p.Val129Met	

Patient (#)	Sex (M/F)	Age (Y)	Platelets (x10^9/L)	INR (ratio)	aPTT(sec)	PT (sec)	Factor IX (%)	Factor XI (%)	Protein C (%)	Protein S (%)	Antithrombin (%)	Genotype	Events
14	M	7		1.0–1.1	31–35		**58–77**	**42–71**	**49–69**	71	**25–43**	p.Arg141His; p.Phe183Ser	
15	F	12	293–303	1.0–1.1	27–32	11.5–12.3	72–107	95–111	94–120	90–91	96–113	p.Arg141His; *	
16	M	10	246–282	1.0–1.1	28–30	11.2–12.4	**63–77**	96–136	72–98	**59–77**	98–122	p.Arg141His; *	Epistaxis
17	F	7	198–258	**1.1–2.0**	**22–50**	**12.0–21.7**	70	**30–96**	**29**	72	**33–147**	p.Pro113Leu; p.Thr237Arg	Easy bleeding, SLE
18	F	19	206		29	**13.9**	100–116	105	93–150	79	102–124	p.Val60Leu; **	
19	F	23		1.1	31							p.Arg141His; p.Pro69Ser	
20	M	10	268–342	1.0–1.1	37	11.2–12.1	72	82–89			133–148	p.Arg141His; p.Arg238His	Easy bleeding, epistaxis, hematochezia
21	M	9	**133–153**	**1.1–1.2**		**12.9–14.4**	**56**	**31–40**	**30**	83	**33–34**	p.Arg141His; p.Val231Met	Easy bruising
22	M	5	420				112	75–106			**59–85**	p.Arg141His; p.Val231Met	Easy bleeding
23	M	10	287–384	1.0–1.1	35–36	10.3–12.4	70–72	**53–72**	**61**	**59**	**47–67**	p.Ser47Leu; p.Gln33Pro	
24	M	17	**167–254**	**1.1–1.2**	**35–38**	**11.7–13.6**	74–76	**22–30**	**46**	70	**27–34**	p.Arg141His; p.Ile153Thr	Perioperative bleeding
25	M	9	258–383	**1.0–1.2**	32.3–37	**13.1–14.7**	68–97	57–61	**35–62**	**54–71**	**48–71**	p.Arg141His; p.Pro113Leu	
26	M	13	230–316	1.0–1.1	32–34	11.5–12.4	74–75	67–82	73–74		86–108	p.Arg141His; p.Cys241Ser	Epistaxis
27	M	8	212–383	**1.2**	27–36	**12.8–12.9**	72–103	**38–61**	**41–57**	81	**40–80**	p.Asp148Asn; p.Pro113Leu	SLE
28	M	14	356–458	**1.0–1.3**	26–32	**11.1–16.0**		**49**			**36**	p.Arg141His; p.Ile132Thr	Hematemesis and hematochezia
29	M	8	238–337	0.9–1.1	**41**	10.3–11.9		58–80	**35**	**40–51**	**45–91**	p.Arg141His; p.Val44Ala	

Patient (#)	Sex (M/F)	Age (Y)	Platelets (x10^9/L)	INR (ratio)	aPTT(sec)	PT (sec)	Factor IX (%)	Factor XI (%)	Protein C (%)	Protein S (%)	Antithrombin (%)	Genotype	Events
30	F	10									**66**	p.Arg141His; Ile110Ser	
31	M	29	209–331	**1.0–1.3**	27–36	**10.8–14.3**	81–95	**28–57**	**30–47**	**44–65**	**14–27**	p.Arg141His; p.Phe119Leu	DVT
32	M	0.5		**1.3**	**45**	**16.1**						p.Arg141His; p.Val231Met	
33	M	1	**108–319**	**1.9**	**50**	**21.7**					**47–82**	p.Arg141His; p.Val231Met	DVT, SLE
34	F	2	392		31	**13.1**	68	**51**	**56**	87	**78**	p.GLy15Arg; p.Ile153Thr	
35	M	4	**29–467**	**1.0–1.3**	**24–42**	**11.5–13.9**	**34–41**	**18–59**	**36–83**		**24–71**	p.Arg141His; p.Phe119Leu	
36	M	26	**61–297**	1.0	26–31	**10.8–13.2**	71–112	**29–58**	**40–60**	**32–44**	**40–48**	p.Phe11–9Leu; *	Perioperative Bleeding, hematochezia, SLE
37	M	3	615–620	1.0	35	**13.0–13.7**					**28**	p.Arg141His; p.Phe119Leu	
38	F	35	188–423	1.0–1.1	**34–38**	**10.5–14.1**	111	**24–68**	**45–57**	107	**24–67**	p.Phe119Leu, p.Phe119Leu	Hematochezia
39	F	8	292–344	1.1	27–35	10.7–12.3		85	**38–76**		**38–88**	p.Arg141His; p.Gly186Arg	
40	M	1.08333									**44**	p.Arg141His; p.Phe119Leu	DVT
41	M	8	**119–401**	**0.9–1.3**	21–35	11.2–12.2	97–119	66–122	83–118		**60–118**	p.Arg141His; p.Phe119Leu	Easy bleeding and bruising, epistaxis
42	M	9		0.9	25–28	9.8–10.2	75		**47–73**	64–66	**39–73**	p.Arg141His; p.Pro113Leu	Easy bruising, SLE
43	F	1.83333	706	**0.89–1.13**	**40–45**	10.0-12.5	**41–81**	**22–38**	**56**	**40–70**	**21–54**	p.Leu243Pro; p.Thr237Arg	
44	F	9	372-408	**1.0–1.2**	31–32	**11.0–13.6**	100–109	63–77	84		**51–75**	p.Asp188Gly; p.Val231Met	SLE

Patient (#)	Sex (M/F)	Age (Y)	Platelets (x10^9/L)	INR (ratio)	aPTT(sec)	PT (sec)	Factor IX (%)	Factor XI (%)	Protein C (%)	Protein S (%)	Antithrombin (%)	Genotype	Events
45	M	3		0.9–1.1	32–37	**11.8–13.5**					**47–57**	p.Gln37Ter; p.Pro113Thr	
46	M	9	333–357	1.0	24–27	10.7–11.1	83–98	108–110	120–121		92–107	p.Pro69Ser; p.Asp148Asn	
47	M	14	303–357	0.9–1.1		**11.9–13.2**		**52–70**	94		**59–72**	p.Phe157Ser; p.Thr237Met	
48	F	24	202–281	1.0–1.1	29–32	10.9–11.9	89–97	89–109	137–170	100	96–128	p.Arg123Gln; p.Cys241Ser	Hematemesis, SLE
49	M	25	299–346	**1.2**	30–35	**12.7–13.6**	97	**50–66**	**50–52**		**35–47**	p.Cys9Tyr; p.Asp148Asn	
50	M	8	302–339	1.0–1.1	26–31	**9.7–13.8**	79–115	**45–65**	**48–65**		**46–60**	p.Arg141His; p.Asn216Ile	

Hemostasis values are displayed as ranges for each patient (lowest value measured-highest value measured). The hemostatic values of patients who had at least one abnormal result are displayed in bold.

Reference values—platelets: 187–400 ×10^9/L; international normalized ratio (INR): 0.9–1.1; activated partial thromboplastin time (aPTT): 25–37 seconds; prothrombin time (PT): 9.4–12.5 seconds; factor IX activity: 65–140%; factor XI activity: 55–150%; protein C activity: 70–150%; protein S activity: 60–140%; antithrombin activity (AT): 80–130%.

Abbreviations: M, male; F, female; Y, years; DVT, deep venous thrombosis; SLE, stroke-like episode.

* Intronic—P15: c.640–23A>G, P16: c.640–23A>G, P36: c.639–15479C>T

** Splice—P18: c.255+1G>A

Abbreviations: international normalized ratio (INR), activated partial thromboplastin time (aPTT), prothrombin time (PT)

Table 3.

Summary of bleeding, thrombotic, and other events (possibly related to coagulation abnormalities) in all patients with symptomatic coagulation abnormalities.

Vascular events	Patient ID	Age at time of event (Years)	Sex (M/F)	Genotype	NPCRS
Easy bleeding/bruising	4	4	F	p.Arg141His; p.Phe68Cys	31
	10	5	F	p.Thr237Arg; p.Cys241Ser	17
	17	4	F	p.Pro113Leu; p.Thr237Arg	31
	20	6	M	p.Arg141His; p.Arg238His	17
	21	9	M	p.Arg141His; p.Val231Met	26
	22	5	M	p.Arg141His; p.Val231Met	26
	41	3/4 - 2	M	p.Arg141His; p.Phe119Leu	34
	42	9	M	p.Arg141His; p.Pro113Leu	20
Epistaxis	16	11	M	p.Arg141His; *	26
	20	6	M	p.Arg141His; p.Arg238His	17
	26	4 & 11	M	p.Arg141His; p.Cys241Ser	14
	41	1	M	p.Arg141His; p.Phe119Leu	34
Excessive bleeding - perioperatively	1	2	M	p.Gly15Ala; p.Arg141His	20
	12	3/4	M	p.Arg141His; p.Asn216Ile	29
	24	15	M	p.Arg141His; p.Ile 153Thr	22
	36	15	M	p.Phe119Leu; *	33
Hematemesis/hematochezia	20	6	M	p.Arg141His; p.Arg238His	17
	28	2	M	p.Arg141His; p.Ile132Thr	23
	36	15	M	p.Phe119Leu, p.Phe119Leu	33
	38	21	F	p.Arg141His; p.Val231Met	22
	48	10	F	p.Arg123Gln; p.Cys241Ser	9
Deep venous thrombosis	1	3	M	p.Gly15Ala; p.Arg141His	20
	12	3/4	M	p.Arg141His; p.Asn216Ile	29
	31	20 & 29	M	p.Arg141His; p.Phe119Leu	29
	33	3/4	M	p.Arg141His; p.Val231Met	37

Vascular events	Patient ID	Age at time of event (Years)	Sex (M/F)	Genotype	NPCRS
	40	Neonatal	M	p.Arg141His; p.Phe119Leu	21
Other events	**Patient ID**	**Age at time of event (Years)**	**Sex (M/F)**	**Genotype**	**NPCRS**
Stroke-like episode	8	4	M	p.Pro113Leu; pVal182Asp	19
	10	7	F	p.Thr237Arg; p.Cys241Ser	17
	17	3 & 4	F	p.Pro113Leu; p.Thr237Arg	31
	27	4	M	p.Asp148Asn; p.Pro113Leu	27
	33	1/2	M	p.Arg141His; p.Val231Met	37
	36	17 & 21 & 24	M	p.Phe119Leu; *	33
	42	8	M	p.Arg141His; p.Pro113Leu	20
	44	5	F	p.Asp188Gly; p.Val231Met	25
	48	5	F	p.Arg123Gln; p.Cys241Ser	9

Abbreviations: deep venous thrombosis (DVT), stroke-like episode (SLE), Nijmegen Patient CDG Rating Scale (NPCRS)

* Intronic—P16: c.640–23A>G, P36: c.639–15479C>T