MPI-CDG with transient hypoglycosylation and antithrombin deficiency

Antithrombin deficiency is a strong risk factor for venous thromboembolism (VTE) whose testing has demonstrated usefulness.[1-5] Most cases of antithrombin deficiency are explained by mutations in *SERPINC1*, the gene encoding this anticoagulant, with detection rate ranging from 70-80%.[6,7] In about 20-30% of cases without *SERPINC1* gene defect, the underlying mechanism was hypoglycosylation.[8] An aberrant N-glycosylation causing a recessive or transient antithrombin deficiency is a new form of thrombophilia which is still underestimated.[8] Congenital disorders of glycosylation (CDG), a rare disease, are actually usually associated with antithrombin deficiency.[9] The relevance of glycosylation in multiple proteins and systems explains why CDGs have multiorgan defects including psychomotor delay. However, MPI-CDG, caused by mutations in phosphomannose-isomerase gene (*MPI*), is one of the few CDG with or without minor neurological involvement.[9] MPI-CDG patients suffer from protein-losing enteropathy, hypoglycemia and congenital hepatic fibrosis, and all have antithrombin deficiency.[10] Only 19 pathogenic variants in MPI have been described (*http://www.hgmd.cf.ac.uk/ac/all.php*).

A 35-year-old woman had loose stools with laboratory signs of inflammation and hypoproteinemia since the age of 12. The initial diagnosis was inflammatory bowel disease and the patient was treated with oral 5-aminosalicylic acid compounds and corticosteroids. Due to symptoms of ileus, the patient required surgery. Crohn's disease established at the age of 16 based on the 80-centimeter long resected ileum examination which revealed

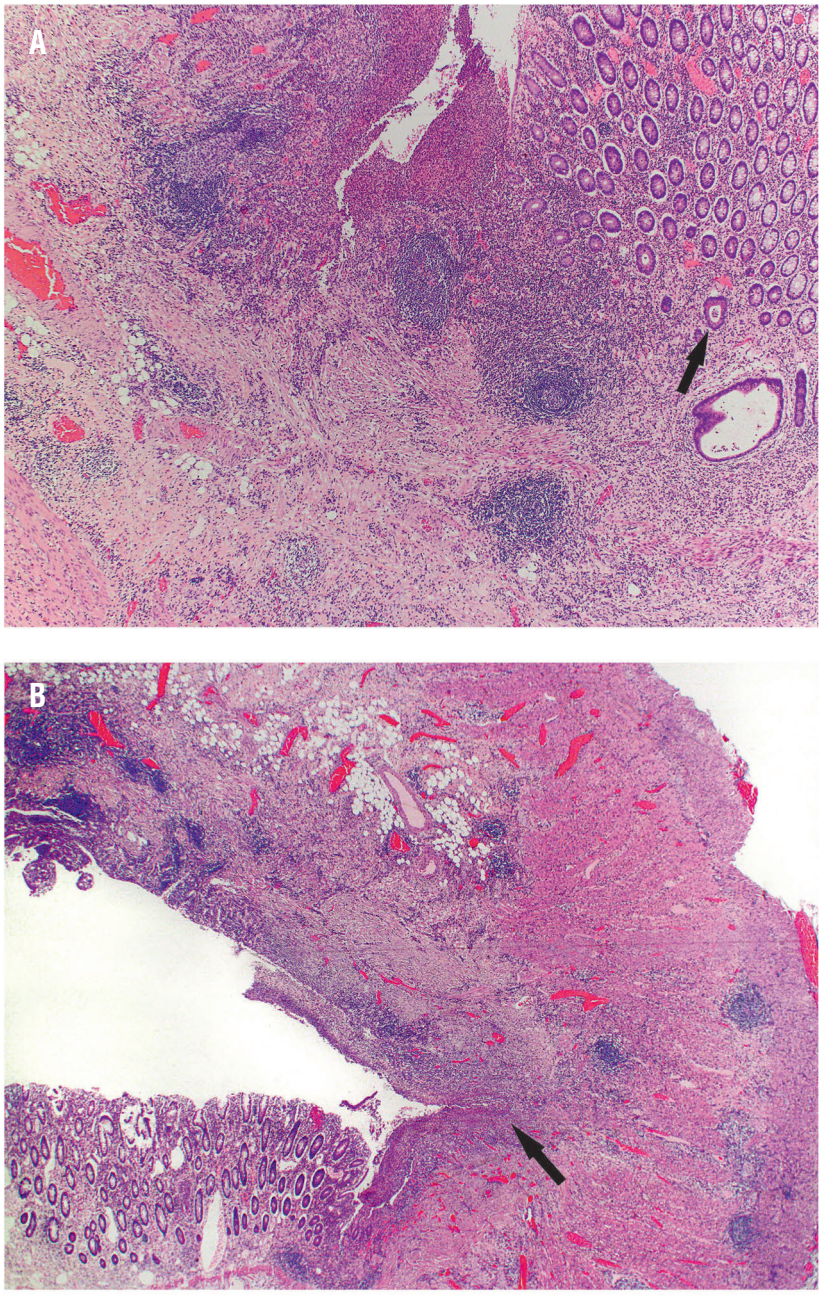

Figure 1. Histological analysis (hematoxylin-eosin staining) of the proband intestinal biopsy. A. Marked intestinal wall thickening with ileal stenosis. Ulceration (arrow) and lymphocyte infiltration (magnification 5x). B. Ulceration with granuloma and active inflammation. A small abscess (arrow) (magnification 5x).

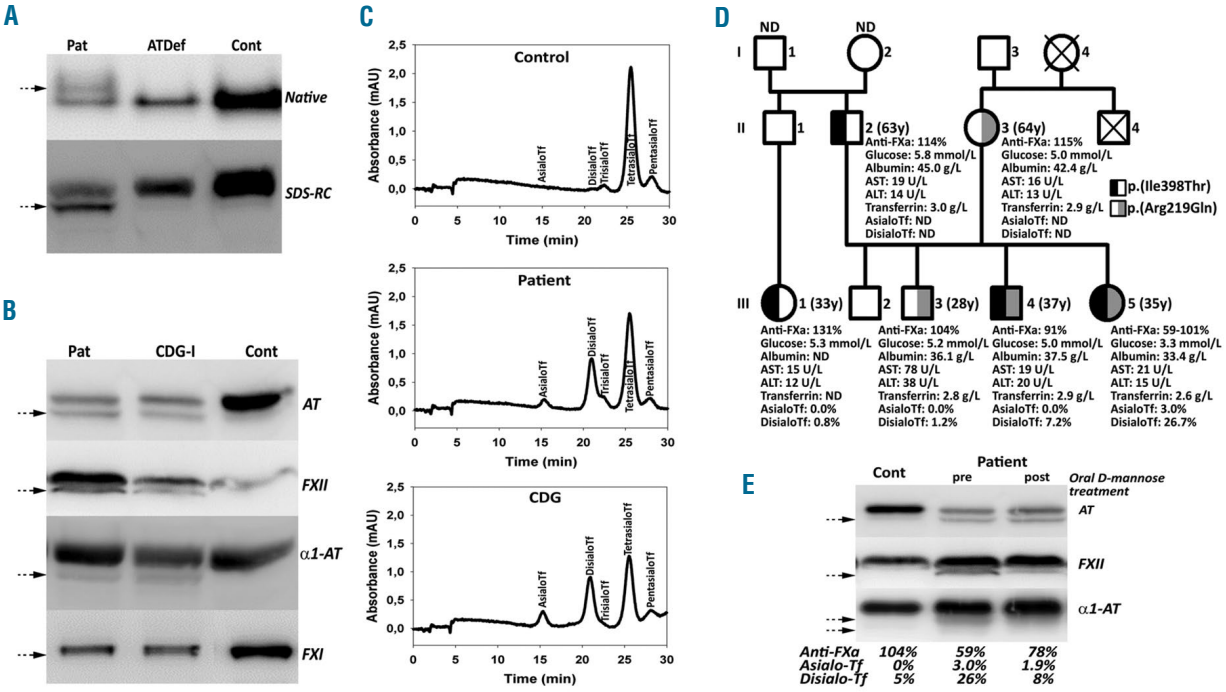

Figure 2. Characterization of MPI-CDG in the proband and her family. A. Identification of aberrant antithrombin in plasma of the proband (Pat). A patient with type I antithrombin deficiency caused by a non-sense variant (AT Def) and a pool of 100 healthy subjects (Control) were used as controls. Antithrombin was immunodetected with a specific polyclonal antibody after native or SDS polyacrylamide gel electrophoresis (PAGE) of plasma. Hypoglycosylated antithrombin is indicated by dashed arrows. B. Electrophoretic pattern of antithrombin (AT), FXII, FXI and α1-antitrypsin (α1-AT) in the proband (Pat), a PMM2-CDG patient (CDG-I) and a pool of plasma from 100 healthy subjects (Cont). Detection was done after SDS-PAGE with specific polyclonal antibodies. Hypoglycosylated proteins are indicated by dashed arrows. C. HPLC pattern of transferrin glycoforms in the proband, a PMM2-CDG patient (CDG-I) and a pool of plasma from 100 healthy subjects (Control). D. Family tree of the proband (III-5, indicated by an arrow). The *MPI* p.Ile398Thr gene variant is indicated by black symbols, and the p.Arg219Gln variant by gray symbols. The proband's mother (II-3) with diabetes mellitus type 2 (DM2) experienced numerous incidents of hypoglycemia. Her brother (II-4), also with DM2, died suddenly at the age of 62. The proband's grandmother (I-4) died of pancreatic cancer at the age of 54. The proband's older brother (III-4) was diagnosed with gastritis caused by *Helicobacter pylori* and reported intestinal malabsorption, with a long history of loose stools and bloating, but gastroscopy showed no abnormalities. The proband's younger brother (III-3) suffered from DM2. Blood samples of the oldest brother (III-2) were unavailable for analysis. E. Evolution of antithrombin anticoagulant activity (Anti-FXa) and levels of asialo and disialo transferrin (Tf) in the proband after treatment with D-mannose. The variation of hypoglycosylated forms of antithrombin (AT) factor XII (FXII) and α1-antitrypsin (α1-AT) (pointed by dashed arrows) caused by this treatment is also shown.

typical histopathological findings (Figure 1) and classic endoscopic and radiological criteria.[11] Due to perianal fistulas at the age of 22, treatment with infliximab 5 mg/kg body weight and prednisone 40 mg daily was introduced. After 4 doses over 3 months, she experienced high-risk pulmonary embolism (PE) with flowing thrombi visualized in the right atrium and underwent surgical embolectomy. The postoperative period was uneventful. Thrombophilia screening showed reduced antithrombin activity (Anti-FXa= 59%, reference range [N]: 75-125%) combined with antigen levels in the low normal range (nephelometry, 0.198 g/L, [N]: 0.19-0.31 g/L), which led to the diagnosis of antithrombin deficiency. Deficiency of protein C and S was excluded. Fibrinogen, factor VIII, antiphospholipid antibodies were normal. Factor V Leiden, G20210A prothrombin variant and lupus anticoagulant were absent. The proband also had asymptomatic hypoglycemia (minimum 3.3 mmol/L) with normal insulin concentrations (11.9 μU/ml) and normal liver function (aspartate aminotransferase: 21 U/L; alanine transaminase, 15 U/L). Family history of VTE was negative. The antithrombin activity of all family members was >90%. Anticoagulant treatment with acenocoumarol (target INR 2-3) was initiated.

Five months after PE, immunosuppressive treatment with azathioprine 100 mg daily followed by adalimumab was administered. However, within a few weeks she developed abdominal abscess requiring surgical drainage followed by another disease exacerbation after 17 months treated with parenteral nutrition. After the subsequent one-year adalimumab treatment, clinical remission was achieved, however, elevated inflammatory markers and hypoproteinemia persisted. When the perianal fistula recurred she received metronidazole with symptom resolution. On admission to the Center for Coagulation Disorders in 2014, the patient reported weakness, dizziness, easy bruising, perianal pain associated with painful defecation and loose stools ten times a day. A control analysis showed antithrombin activity and unexpectedly normal antigen levels (101% and 0.237 g/L, respectively). One month later, based on patient preferences, when the control antithrombin was again within the reference range (83%), the patient discontinued oral anticoagulation. Due to increased gastrointestinal signs and symptoms, she underwent perianal fistula surgery and treatment with oral mercaptopurine 50 mg daily and mesalamine 2 g daily followed. Twenty-four months later, antithrombin activity using a thrombin-based assay

was 75% ([N]: 79-112%), and FXI: 68% ([N]: 70-120%).

The diagnosis of inherited antithrombin deficiency made 9 years before was questioned. No relevant *SERPINC1* genetic defects were detected by sequencing exons and flanking regions, as well as 1500 bp of the promoter region and MLPA. Intriguingly, the Western blot of plasma antithrombin from a sample displaying its low anti-FXa values (58%) revealed increased levels of a variant antithrombin (Figure 2A). The electrophoretic pattern was identical to that of CDG (Figure 2B), supporting a hypoglycosylated antithrombin. Increased levels of hypoglycosylated forms of other proteins were also detected and their pattern was identical to that observed in CDG (Figure 2B). This study also confirmed the FXI deficiency, like CDG patients (Figure 2B). Finally, HPLC analysis of transferrin glycoforms showed increased levels of asialo- and disialo-transferrin and thus confirmed the diagnosis of CDG (Figure 2C,D). Mild increase of disialo-transferrin was also found in the proband's older brother who had anti-FXa= 91% and also had enteropathy (Figure 2D).

Sequencing of 75 genes involved in the N-glycosylation pathway[12] revealed two pathogenic mutations in *MPI*: c.1193T>C; p.Ile398Thr and c.656G>A; p.Arg219Gln. These variations were verified by Sanger sequencing and also detected in her older brother (Figure 2D).

Based on previous reports in MPI-CDG patients,[13,14] the patient was advised to use supplementation of 700 mg D-mannose twice daily taken during meals. One month after introducing the mannose, the patient reported improvement of the stool consistency and number. At the same time, moderate increase in antithrombin and FXI were observed, 78% and 70%, respectively, and showed reduction of hypoglycosylated forms of antithrombin and transferrin, although they were still detectable. The patient was advised to increase dose of D-mannose (Figure 2E) to 1400 mg twice daily (maximum recommended daily dose: 8000 mg).[13,14] Three months later, the patient reported better exercise tolerance and significant improvement of gastrointestinal symptoms without any adverse events. Her brother, with identical pathogenic variants, was also put on D-mannose 700 mg bid and reported improved gastrointestinal symptoms.

We presented two MPI-CDG related adult cases with protein losing enteropathy, transient antithrombin deficiency and unprovoked PE only in one case, caused by biallelic compound heterozygosity for MPI p.(Ile398Thr) and p.(Arg219Gln) variations.

These variants were described separately in three pediatric patients with established diagnosis of MPI-CDG.[13,15,16] Two unrelated patients carried the p.(Arg219Gln) variant.[13,16] Clinical symptoms in the first p.(Arg219Gln) carrier began at the age of 11 months with diarrhoea and vomiting followed by enteropathy, and recurrent thrombosis.[13] Two additional sibs carriers of p.(Arg219Gln) in biallelic compound heterozygosity with p.(Ile140Thr) reported early recurrent venous thrombosis, enteropathy and hepatic fibrosis (proved by biopsy in the sib who died at age 5).[11] The surviving MPI-CDG patient had no further symptoms following childhood, and antithrombin gradually increased to reach normal levels at age 7, but low values were also reported over time (52-73%).[11] The carrier of the second variant p.(Ile398Thr), in biallelic compound heterozygosity with p.(Tyr255Cys), was referred to the hospital for seizures related to hypoglycemia associated with increased insulin levels at the age of 3 months. She suffered from hypotonia, firm hepatomegaly and vomiting. In the hospital she required parenteral nutrition because of intractable diarrhea, and she had right auriculothrombosis.[15] In all these MPI-CDG patients, daily oral mannose administration turned out to be successful therapy, which corrected the biochemical phenotype although never reaching normal values, and significantly improved clinical signs.[13,15,16]

In the current proband and her brother, one might expect at least similar clinical manifestations. However, they did not suffer from any disorders in their infancy and childhood. The prevalent manifestations were loose stools, which led to the diagnosis of Crohn's disease in the proband. Given histological findings, the diagnosis appeared appropriate despite a relatively poor response to biologic therapy. Based on literature, we suspected that the MPI-CDG detected in the proband manifested as protein-losing enteropathy with loose stool, low BMI (19 kg/m^2), and hypoalbuminemia (25.9 g/L) could mimic Crohn's disease. However, available evidence suggests that inflammation of the intestinal wall, typical of Crohn's disease, with fistulas and abscesses, along with the signs and symptoms reported from the age of 12, leads to the firm diagnosis of Crohn's disease, which coexists with enteropathy associated with MPI pathogenic variants reported in affected children.[13,15,16]

The current case, along with the absence of VTE in her older brother with the same variants, suggests that a prothrombotic state related to MPI-CDG was shifted toward symptomatic high-risk PE by the concomitant Crohn's disease and possibly by the medications used to treat it. Indeed, the inflammatory bowel disease itself increases the risk of VTE with a rate of 6.3 per 1,000 person-years,[17] and corticosteroids used to treat Crohn's disease, rather than anti-TNF monoclonal antibodies, increase the risk of thrombosis.[17]

The second remarkable finding in this family was the normal antithrombin and the low hypoglycosylation observed in the brother carrying both MPI variants and, at some time points, in the proband. This observation, also made previously for other adult MPI-CDGs,[11,16] suggests that phenotypes of *MPI* pathogenic variants are highly heterogeneous and likely modulated by poorly defined factors. Westphal and coworkers suggest that the cellular import of mannose, the activity of phosphoglucose isomerase, or diet variations affecting mannose availability could be involved in the final hypoglycosylation defect and deficiency of multiple proteins including antithrombin,[16] which might also account for the onset of clinical consequences.

Mannose treatment in our cases raises antithrombin levels and alleviates the gastrointestinal symptoms *ex iuvantibus*, thus confirming a significant contribution of the MPI variations to the clinical phenotype, particularly in terms of gastrointestinal manifestation, and supports the usefulness of this treatment also for adult MPI-CDGs, in contrast to previous suggestions.[16]

Despite the low number of cases described worldwide, the clinical characteristics of this disorder, especially the absence of psychomotor retardation, and its diagnosis in patients who have never experienced any clinical manifestations,[18] or with unexpected clinical and phenotypic heterogeneity (this report) led to suggest that MPI-CDG could be underestimated. Screening MPI variants among VTE patients with low antithrombin without pathogenic variants in the *SERPINC1* gene[8] as well as among patients with inflammatory bowel disease who had a poor response to biological treatments is also recommended because these patients may benefit from simple, useful and safe treatment with oral D-mannose.

María Eugenia de la Morena-Barrio,[1] Ewa Wypasek,[2] Danuta Owczarek,[3] Antonia Miñano,[1] Vicente Vicente,[1] Javier Corral[1] and Anetta Undas[4]

[1]Servicio de Hematología y Oncología Médica, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, Spain; [2]John Paul II Hospital, Faculty of Medicine and Health Sciences, Andrzej Frycz Modrzewski Krakow University, and the Institute of Cardiology, Jagiellonian University Medical College, Poland; [3]Department of Gastroenterology, Hepatology and Infectious Diseases, Jagiellonian University Medical College, Kraków, Poland and [4]Institute of Cardiology, Jagiellonian University Medical College, John Paul II Hospital, Kraków, Poland

MEMB and EW contributed equally to this work.

Correspondence: javier.corral@carm.es
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