Received: 26 April 2020 | Revised: 27 June 2020 | Accepted: 15 July 2020

DOI: 10.1002/jimd.12289

ORIGINAL ARTICLE

JIMD SSIEM WILEY

Long term outcome of MPI-CDG patients on D-mannose therapy

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Abstract

Mannose phosphate isomerase MPI-CDG (formerly CDG-1b) is a potentially fatal inherited metabolic disease which is readily treatable with oral D-mannose. We retrospectively reviewed long-term outcomes of patients with

Funding information
ANR, Grant/Award Number: ANR-15RAR3-0004-06; ERA-net, Grant/Award Number: N°643578

Communicating Editor: Eva Morava

MPI-CDG, all but one of whom were treated with D-mannose. Clinical, biological, and histological data were reviewed at diagnosis and on D-mannose treatment. Nine patients were diagnosed with MPI-CDG at a median age of 3 months. The presenting symptoms were diarrhea (n = 9), hepatomegaly (n = 9), hypoglycemia (n = 8), and protein loosing enteropathy (n = 7). All patients survived except the untreated one who died at 2 years of age. Oral D-mannose was started in eight patients at a median age of 7 months (mean 38 months), with a median follow-up on treatment of 14 years 9 months (1.5-20 years). On treatment, two patients developed severe portal hypertension, two developed venous thrombosis, and 1 displayed altered kidney function. Poor compliance with D-mannose was correlated with recurrence of diarrhea, thrombosis, and abnormal biological parameters including coagulation factors and transferrin profiles. Liver fibrosis persisted despite treatment, but two patients showed improved liver architecture during follow-up. This study highlights (i) the efficacy and safety of D-mannose treatment with a median follow-up on treatment of almost 15 years (ii) the need for life-long treatment (iii) the risk of relapse with poor compliance, (iii) the importance of portal hypertension screening (iv) the need to be aware of venous and renal complications in adulthood.

KEYWORDS
coagulation, congenital disorder of glycosylation, congenital hepatic fibrosis, diarrhea, D-mannose, MPI-CDG, portal hypertension

1 | INTRODUCTION

Congenital disorders of glycosylation (CDG) are a group of inherited multisystem disorders characterized by abnormal glycosylation of proteins. MPI-CDG is due to autosomal recessive mutations in the *MPI* gene encoding Mannose Phosphate Isomerase (MPI), a cytosolic enzyme transforming Fructose-6-Phosphate to Mannose-6-Phosphate in the GDP-mannose biosynthesis pathway. The first descriptions of MPI-CDG patients in the 1980s reported a severe protein losing enteropathy responsible for early death.[1] Other symptoms included thrombosis,[1-3] polycystic kidneys,[4,5] and hyperinsulinism.[6] Intrafamilial heterogeneity[2] as well as incidental diagnosis in adulthood without liver or intestinal involvement have also been described.[7,8]

MPI-CDG can be fatal without treatment. Survival dramatically improved with the introduction of D-mannose treatment in 1998.[9-13] However, liver disease persists on treatment and complications may occur despite therapy.[14] Long term follow-up on D-mannose has not been described in the literature. To better delineate the long-term outcomes of MPI-CDG patients on treatment, we reviewed clinical and biochemical data of nine patients with a median follow-up of more than 14 years (2y4m-20y4m), the oldest patient being 26 years old.

2 | PATIENTS AND METHODS

We included all patients with MPI-CDG followed since 1991 in francophone reference centers for metabolic and liver diseases. The diagnosis of MPI-CDG was based on abnormal CDG-I glycosylation pattern of serum glycoproteins by Western blotting assay, a decreased cytosolic MPI activity in blood mononucleated leukocytes,[15] and the presence of bi-allelic *pathogenic variants* in the *MPI* gene. The following data were collected retrospectively from medical records: clinical, biological, and oral D-mannose treatment modalities. Liver and intestinal biopsies were reviewed. Liver tissues were fixed in 4% acetic formalin, embedded in paraffin, cut into 4-mm sections, and stained with hematoxylin and eosin (H&E). Perls' reaction, reticulin, Sirius Red, and cytokeratin 7 (CK7) staining were also performed. The METAVIR score was used for grading portal fibrosis from F0 (no fibrosis) to F4 (cirrhosis). Liver stiffness (ARFI and Transient elastography) data were

reviewed. The study was approved by the local institutional ethical committee at Necker Children's Hospital and each participating center, respectively.

3 | RESULTS

Nine children (five females) were included. Their characteristics are summarized in Tables 1 and 2. One patient died (B) at 2 years of age, when the disease was established, and the treatment not initiated. The remaining eight patients were alive on oral D-mannose, with a median follow-up of 14 years 10 months (patients age: 2y6m-26y8m).

3.1 | Initial presentation

Table 1 summarizes clinical symptoms and Table S1 summarizes biological data. Patients presented between 10 days and 6 months of life (median 3 months). The MPI-CDG diagnosis was confirmed at ages spanning from 1.5 months to 10.5 years (median 7 months). Patients with late diagnosis were either born before 1998 when MPI deficiency was first identified,[11] or came from countries with limited diagnostic resources. All patients presented with hepatomegaly and severe diarrhea at diagnosis, while eight had hyperinsulinemic hypoglycemia and seven protein-loosing enteropathy and hypoalbuminemia. Splenomegaly (SM) was absent at diagnosis but appeared during the first year of life in six patients. Transaminases and GGT levels were mildly elevated in six patients while bilirubin levels were always normal. Platelet count was normal in all patients despite SM but decreased during follow up in 3/6 patients with portal hypertension. Factor V and prothrombin time were decreased in four patients. When performed, factors IX and XI were very low in four, as well as anti-thrombin III and protein C and S levels in three (Table S1). Anemia was identified in five patients.

3.2 | Outcome on D-mannose treatment

Clinical and biological parameters on D-mannose therapy are summarized in Tables 1, 2 and Table S1. Treatment was initiated at a median age of 7 m, immediately following diagnosis. The follow-up on treatment ranged from 2 years 4 months to 20 years 4 months (median 14y9m). Intake modalities of oral D-mannose are specified in Table 1. No side effects was observed. After D-mannose initiation, hypoglycemia and severe diarrhea resolved in all patients within 1 week and in less than 2 weeks, respectively. Five patients, presented with recurrent episodes of diarrhea which were associated with poor compliance of D-mannose treatment, viral infection or severe portal hypertension. In those patients who had factors IX and XI, anti-thrombin III and proteins C and S measurements, levels increased on treatment and decreased with poor compliance (Table S1).

Liver involvement and complications: Liver involvement and portal hypertension persisted for some patients despite D-mannose therapy. Transaminases and GGT normalized on treatment except in three patients who developed portal hypertension (D,F,G). Prothrombin time normalized within 10 days in all patients. However, a decrease in clotting factors was observed later in one patient with hypersplenism (G). All patients had hepatomegaly at diagnosis including the earliest presentation (10 days), but none went on to develop jaundice or liver failure. Six (6) patients (B,D,F,G,H,I) developed splenomegaly after hepatomegaly before 1 year of age except for the patient diagnosed at the age of 6 years (I). The three patients without SM (A,C,E) showed no evidence of portal hypertension and liver stiffness at ages 14, 18, and 24 was normal.

Portal hypertension: Among the six patients with SM, three (B,H,I) had a normal platelet count at last follow-up: one died early (B), two (H, I) had abnormal liver stiffness at 6 years and 1.5 years of age, indicating liver involvement without portal hypertension and no esophageal varices (OV) at endoscopy. The three other patients with SM had increased liver stiffness and developed thrombocytopenia (G,F,D), respectively after 1, 2, and 4 years. One patient (D) did not develop OV probably because of a large spontaneous porto-caval shunt; the two others (F, G) developed OV (respectively after 8 and 12 months) and underwent prophylactic endoscopic band ligation (respectively after 5y3m and 1y7m). G developed recurrence of protein-loosing enteropathy with refractory ascites ascribable to severe portal hypertension, and requiring total parenteral nutrition. He finally underwent surgical mesenterico-caval shunt procedure at 4 years of age which corrected the symptoms. He later developed a large hepatocellular adenoma with a high risk of bleeding and malignant transformation and is currently awaiting liver transplant (12.5 years). None of the patients presented with cardiac or pulmonary complication linked to portal hypertension.

Thrombotic complications: Among patients who had clotting factors measured, Prothrombin time, factors V, IX, and XI, proteins C and S were low at diagnosis (Supplementary Table S1) but tended to normalize on treatment, except in those patients with poor compliance or in the one who developed portal hypertension and hypersplenism (G). In one patient (I) factor V was very

TABLE 1 Characteristics of patients: clinical, *PMI* mutations, enzyme activity, and D-mannose treatment

	Patients	A	B[a]	C	D[a]	E[a]	F	G	H	I
	Year of birth	1991	1992	1993	1997	2001	2005	2007	2009	2015
	Age at last follow-up	26y8m	2y7m (died)	24y6m	20y8m	17y1m	12y9m	12y4m	8y5m	2y6m
	Gender	M	M	F	F	F	F	M	F	M
Initial presentation	Age at onset of clinical manifestations	6m	3m	3m	3m	20d	3m	2m	6m	10d
	Age at diagnosis	10y6m	2y	6y4m	3m	4m	7m	6m	6y10m	1m1/2
	Hepatomegaly	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Splenomegaly	No	10m	No	3m	No	7m	4m	6y	3m
	Diarrhea	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Protein-loosing enteropathy	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
	Hypoglycemia	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
	Other		Skin angioma		IVC					PDA
PMI mutations and enzymatic activity	Consanguinity	No	No	No	No	No	No	No	Yes	No
	PMI activity (U/g; N>5,5)	1.2	0.2	0.8	2	0.3	0	0	nd	0
	Base change allele 1	c.842_844del	c.304C>T	c.386A>G	c.764A>G	c.884G>A	c.655C>T	c.973delC	c.1193T>C	c.884G>A
	Protein change allele 1	p.Gly281del	p.Ser102Leu	p.Tyr129Cys	p.Tyr255Cys	p.Arg295His	p.Arg219Trp	p.Leu325Serfs*24	p.Ile398Thr	p.Arg295His
	Base change allele 2	c.466G>A	c.413T>C	c.455G>A	c.1193T>C	c.884G>A	c.1252C>T	c.1193T>C	c.1193T>C	c.884G>A
	Protein change allele 2	p.Glu156Lys	p.Met138Thr	p.Arg152Gln	p.Ile398Thr	p.Arg295His	p.Arg418Cys	p.Ile398Thr	p.Ile398Thr	p.Arg295His
D-Mannose treatment/ Transferrin profile	Age at start of D-Mannose	10y6m	na	6y4m	4m	4m	8.5m	6m	6y10m	1.5m
	Duration of treatment	16y2m	na	18y2m	20y4m	16y9m	12y	11y10m	1y7m	2y4m
	Initial transferrin profile	Abnormal	na	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal
	Transferrin profile over time	Normal	na	Variable	Variable	Normal	Normal	Normal	Normal	Normal
	Mannose treatment (g/kg/d)[b]	0.4-1.8	na	0.25-0.30	0.2-0.5	0.4-0.7	0.5-1	0.6-1.2	0.2–0.5	0.8-1.6
	Mannose T0 (μmol/L)	24	na	35	36	31	na	32	na	71
	Mannose Peak (μmol/L)	200	na	256	202	158	na	164	na	188
	Compliance	Variable	na	Poor	Poor	Poor	Good	Good	Good	Good

Abbreviations: d, day; F, female; IVC, intra ventricular communication; m, month; M, male; na, not available; nd, not done; PDA, patent ductus arteriosus; y, year.
[a]Patients already published (References [26, 27]).
[b]The spacing intake of mannose during the day was 3 to 5 time per day.

TABLE 2 Clinical evolution of the patients on D-mannose treatment and radiological findings

	Patients	A	B	C	D	E	F	G	H	I
	Age at last follow-up	26y8m	2y7m (died)	24y6m	20y8m	17y1m	12y9m	12y4m	8y5m	2y6m
	Age starting D-Mannose	10y6m	/	6y4m	4m	4m	8m1/2	6m	6y10m	1m1/2
Clinical evolution at last evaluation	Diarrhea	resolved	persists	resolved	variable	variable	variable	variable	resolved	resolved
	Hypoglycemia	resolved	Transient	resolved	resolved	resolved	resolved	resolved	resolved	resolved
	HM	6m	5m	Neonatal	3m	4m	3m	2m	6y	1m
	SM	No	10m	No	3m	No	7m	4m	6y	4m
	Platelets count $10.^9$/l	313	542	391	75	294	104	58	282	332
	Endoscopy	-	-	-	No OV (17y10m) Gastritis	No OV (14y) No gastritis	OV (1y) Gastritis	OV (8m) Gastritis	No OV (6y)	No OV (2y6m)
	Portal hypertension management	-	-	-	-	-	Ligation program (5a3m)	Ligation program (1y7m) Surgical derivation (4y7 m)	-	-
	Hematologic status	Chronic anemia	Chronic anemia Jugular thrombosis (11m) Variable coagulation	Chronic anemia Phlebitis (15y, 17y, 18y, 22y) Pulmonary embolism (24y)	Chronic anemia Variable coagulation Auricular thrombosis (4m)	Chronic anemia	Chronic anemia	Chronic anemia Variable coagulation	Chronic anemia	Chronic anemia
	Puberty	Normal	/	Normal	Delayed (>15y)	Delayed (>14y)	Normal	na	na	na
	Cardiac status	Normal	Normal	Normal	IVC	Normal	Normal	Normal	Normal	PDA
	Renal status	Normal	Hyperechogenic (2y)	Normal	Hyperechogenic (3m), renal function test N (15y)	Clearance 73 mL/min/ 173 m^2 (14y)	Normal	Hyperechogenic (1y), clearance 193 mL/mi/m^2 (4y) and then normal function (7y)	Normal	Normal
	Height SD	0 DS	−3 DS	−2 DS	−1DS	−2DS	−1DS	−1DS	−0.5DS	−3DS
	Neurological status	Normal	Normal	Normal	Normal (IQ 86 at 10y)	Normal (IQ 85 at 7y)	Normal	Delayed walk (3y)	Normal	Normal
Radiological findings	Liver ultrasound (age)	HM	Heterogeneous, Dysmorphic (2y)	HM	Hyperechogeneous (3m), Dysmorphic (2y)	Hyperechogeneous (4y)	Hyperechogeneous (9m), Dysmorphic (2y4m)	Hyperechogeneous (4m), Dysmorphic (1y)	Hyperechogeneous (6y) Dysmorphic (6y)	HM
	Liver MRI (age)	-	-	-	-	-	Dysmorphic (2y8m)	Dysmorphic (3y)	-	-
	Liver stiffness (age) [normal]	3.8 kPa (18y) [<5]	na	6.2 kPa (24y) [<5]	26-49 kPa (17y) 65.2 kPa (19y7m) [<5]	4.6 kPa (14y) [<5]	9 kPa (11y9m) [<5]	33 kPa (8y) 66 kPa (10y) [<5]	2.3 m/s (6y) [<1.1]	24 kPa (3m) 10-29 kPa (1y7m) [<5]
	Spleen (ultrasound)	No SM	-	No SM	SM	No SM	SM	SM	No SM	SM
	Kidney ultrasound (age)	-	Hyperechogenic (2y)	Normal (24y)	Hyperechogenic (3m),	Normal (14y)	Hyperechogenic (9m)	Hyperechogenic (1y)	Normal (6y)	Normal (2y2m)

(Continues)

TABLE 2 (Continued)

Patients	A	B	C	D	E	F	G	H	I
				loss of cortical differentiation (10y)		loss of cortical differentiation (3y7m)			
Heart (ultrasound)	-	Normal	Normal	IVC	Normal	Normal	Normal	Normal	PDA
Brain MRI (age)	-	Hypersignal hemispheric of the cerebellum (2y2m)	-	Hypersignal of posterior horn (3y)	-	-	-	-	-

Abbreviations: abN: abnormal; HM, hepatomegaly; IVC, intra ventricular communication; m, month; N, normal; na, not applicable; No, no; OV, esophageal varices; PDA: persistent ductus arteriosus; SM, splenomegaly; y, year; Y, yes.–: unknown.

low (<15% of normal) at diagnosis without major transaminase elevation, cholestasis or hyperammonemia, and recovered very quickly (less than 72 hours) with treatment. Two patients presented venous thrombosis: one child (D) with a central line and intra-ventricular communication had left intraventricular thrombosis at the age of 4 months which did not relapse on D-mannose. The other (C) presented with four episodes of lower limb thrombophlebitis between 15 and 22 years of age when D-mannose compliance was poor. At age 23 years she became pregnant, and D-mannose was stopped at 8 weeks' gestation because of concern about embryonic lethality possibly related to D-mannose.[16] Preventive anticoagulation by enoxaparin was introduced and she gave birth to a healthy baby birth at 39 weeks of gestation. D-mannose was re-initiated after delivery combined with a preventive oral anticoagulation treatment. She presented with pulmonary embolism 1 year later after discontinuing both treatments.

Other associated features are presented in Table 2. Chronic anemia was seen in two patients (F,G), who had portal hypertension with OV, but no iron deficiency. Cardiac features included persistent ductus arteriosus in 1 patient (I) that spontaneously resolved at 2 years of age, and one asymptomatic intra ventricular communication (D). In four patients (B,D,E,G) the initial renal ultrasound evaluation showed hyperechoic kidneys. Renal function tests (urea, creatinine) were normal. When studied, the glomerular filtration rate (iohexol clearance) showed hyperfiltration at 4 years in one patient (G) and was mildly altered in another patient at age 14 years (E). The height curve was below mean for all patients (range −3 to −0.5 SD). Bone maturation evaluated by radiography for the patient I at age 2 years (height − 3 SD) was normal.

Endocrine: Two of five (2/5) girls displayed delayed puberty (E,D) at the ages of 14 and 15 years. However, values for LH, FSH, and oestradiol were normal for age in both patients, and the puberty of one mother was delayed (data not shown). Sex hormone levels were within the normal range of all other tested patients. IGF1 values were very low at the start of the D-mannose treatment for patient I, and then normalized on treatment. For the two patients with delayed puberty, IGF1 values were just below the normal ranges for age between 6 and 8 years and then normalized with good compliance (patient E), and were low at 16 and 17 years old when the D-mannose were poorly taken (patient D) (data not shown).

Neurological examination was normal in six patients. One patient (G) walked at age 3 years which was attributed to malnutrition in the first years of life, but IQ were in the normal range at age 11 years4 months. Two patients (D,E), had mild learning difficulties with IQ at

85 and 86, respectively, but comparable to their siblings. One patient showed a hypersignal of the cerebellum at 2 years2 months (B) and another 3 years old patient displayed a hypersignal of the posterior horn. Patient D developed normally until adulthood, while B died in absence of treatment.

3.3 | Transferrin Western-blot evolution on treatment and D-mannose dosages

The electrophoretic profiles of serum transferrin (Trf) Western-blot (WB) were abnormal at diagnosis in all patients. After initiation of D-mannnose, Trf WB profiles normalized within 4 to 6 months (D,G,I; Figure 1). The recurrence of abnormal Trf WB profile on treatment was associated with poor compliance (A,C,E,D; Figure 1) and recurrence of diarrhea and abdominal pain. Serum mannose dosage monitoring showed a small peak and rapid clearance, indicating the need for several doses per day (data not shown). We did not observe any correlation between mannose dose/daily intake number and Trf WB profile aspect. Median residual D-mannose serum levels ranged from 24 to 71 μmoL/L and peak from 158 to 256 μmoL/L (Table 1).

3.4 | Liver and intestinal histology

Liver: Liver biopsies were performed in seven patients. Two patients with mild liver involvement did not undergo a liver biopsy (A,C). For two patients (B,H) the liver biopsy was performed before D-mannose initiation. For the five other patients (D,E,F,G,I), the liver biopsy was performed within 5 months to 13 years 5 months after the initiation of D-mannose. Two patients underwent two biopsies to evaluate liver fibrosis (E, at 4y10m and 13y5m of D-mannose therapy) and the development of nodules (G, at 4y1m and 12y of D-mannose therapy). In all patients the lesions mimicked "congenital hepatic fibrosis" with "ductal plate malformation" as previously described[1]: marked proliferation of bile ductules with normal cubic or flattened epithelium, anastomosing biliary channels and angulated bile ducts often dilated with inspissated bile or bile plugs in fibrotic portal spaces. Liver biopsies showed irregularly shaped lobules separated and surrounded by portal—portal bridging fibrosis (stage F1-F4 of the METAVIR classification). Irregular nodules of normal hepatocytes showed mild changes in hepatic cord thickness and sinusoidal fibrosis (reticulin and picrosirius staining), with persistent central veins eliminating the diagnosis of cirrhosis. In the two patients

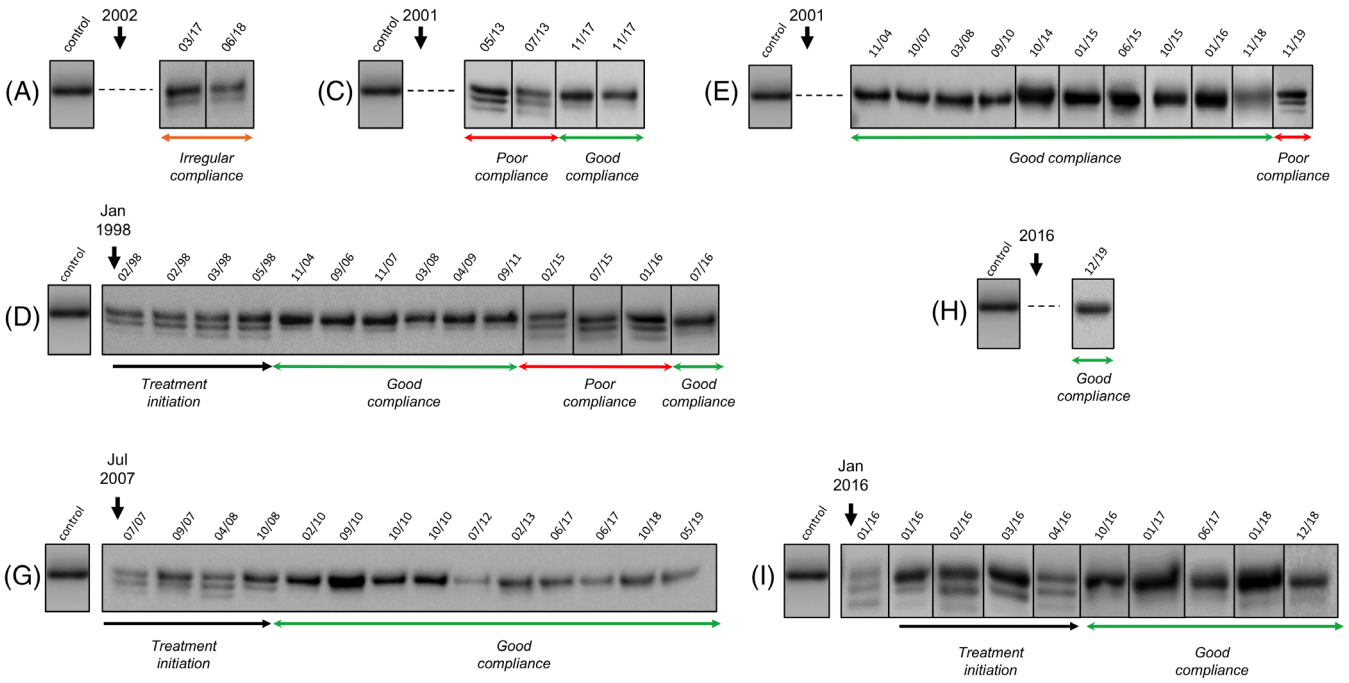

FIGURE 1 Electrophoretic transferrin profile analysis by western-blot (WB Trf) before and on D-mannose treatment over time. Initiation of treatment lead to normalization of the WB profile within a few months in patients D,G,I. Poor compliance to treatment was associated with a recurrence of an abnormal WB profile (A,C,E,D), associated with abdominal pain and diarrhea (E,D) or to late venous thrombosis (C)

who underwent two liver biopsies, it was difficult to conclude regarding progression of portal fibrosis. However, changes of hepatic cord thickness and sinusoidal fibrosis were improved in both patients after prolonged D-mannose treatment (Figure S1).

Intestine: Intestinal biopsies were available for three patients; before D-mannose (B) and on D-mannose (I,D) after 5 months and 2 years 1 month of treatment. In duodenal biopsies the main findings were: normal villi, conserved enterocyte polarity, and lymphatic dilation (lymphangiectasia) in the lamina propria in contact with muscularis mucosa or the villi. Lymphatic dilation (D2-40 staining) was also identified in treated patients (Figure S2).

3.5 | Molecular investigation, MPI enzyme activities, and correlation with phenotype

Data are presented in Table 1. Only one patient originated from a consanguineous family. Blood MPI enzyme activity was performed in eight patients. Enzyme activity was moderately low in two patients (A,D), very low in three (B,C,E), and not measurable in three (F,G,I). In patients with moderately low enzyme activity, one developed a mild liver disease (A) and one had portal hypertension without OV (D). In patients with very low enzymatic activity, two had moderate liver disease (LM,BM) and one died early with a dysmorphic liver (B). In patients with undetectable enzymatic activity, two developed a severe portal hypertension with OV (F, G) and one had a dysmorphic liver with a limited follow-up (I) with portal hypertension which could then appear later. Mutation analysis identified two bi-allelic pathogenic variants in the *MPI* gene (NM_002435) in all patients, accordingly to an autosomal recessive inheritance. Three patients harbored homozygous pathogenic variants and other patients carried compound heterozygous pathogenic variants. Twelve pathogenic variants were identified in the nine patients. They were most often already described missense pathogenic variants, with two pathogenic variants identified in five patients (p.Arg295His and p.Ile398Thr). We identified two new pathogenic variants in two patients with severe liver disease: p.Arg219Trp (F) and p.Leu325Serfs*24 (G). There was no correlation between phenotype and genotype.

4 | DISCUSSION

Long term outcome of MPI-CDG patients on D-mannose remains poorly described, and to date only 26 MPI-CDG successfully treated patients are known.[10] We report here one of the largest series of MPI-CDG patients and our 20-year experience with D-mannose. Like others, we observed a dramatic clinical improvement on D-mannose,[17] but liver fibrosis with portal hypertension persists and remains a long term concern.

Two thirds of our patients presented with a dysmorphic liver or abnormal liver stiffness indicating important architectural changes or portal hypertension. Three patients (D,F,G) developed signs of portal hypertension with OV and two experienced bleeding. One patient (G) presented with severe portal hypertension requiring a surgical meso-caval shunt and leading to development of hepatocellular adenoma; this patient is currently awaiting liver transplantation. There is only one report of a patient with PMI-CDG undergoing liver transplantation for hepatopulmonary syndrome at 28 years of age.[18] We suggest that specialized follow is important to screen for liver disease progression in childhood and adulthood.

We did not observe any correlation between the severity of liver disease and MPI enzyme activity as suggested in the literature.[19] Patients with very low MPI enzyme activity (C, E) had no liver disease or portal hypertension in adulthood, and one with moderately low MPI activity (D) had dysmorphic liver with portal hypertension. We did not identify any correlation between severity of the liver disease and the type of pathogenic variants. Also, asymptomatic forms have been reported with the p.Arg219Trp homozygous mutation and a moderately low MPI activity.[8]

The histological picture of liver disease with "Congenital hepatic Fibrosis" suggests a prenatal onset. Early oral D-mannose supplementation in the first year of life did not prevent liver disease onset as others have suggested.[14,18] Nevertheless, improvement of the lobular architecture and sinusoidal fibrosis was seen in two patients reported here (E,G), suggesting a possible effect of therapy on lobular involvement.

We identified previously undescribed features such as chronic anemia and delayed puberty, and importantly, a normal pregnancy (mannose was stopped at 8 weeks of pregnancy). Like others, we observed delayed growth in most patients. The IGF1 values seem clearly linked to the glycosylation profile and the compliance to D-mannose treatment as already suggested.[20] We also observed hyperechogenic kidney[5] in two patients with an altered renal clearance (E,G). We therefore suggest that renal function should be monitored in adulthood. Developmental delay was not a significant concern.

It was suggested that abnormal glycosylation and its consequences in MPI-CDG patients could change with age explaining adult onset or mild symptoms in adults allowing for D-mannose discontinuation.[2] However, our

observations encourage us to promote life-long treatment with D-mannose in MPI-CDG patients. Indeed, poorly compliant patients (C,D,E) experienced recurrence of abdominal pain, diarrhea and thrombosis, and abnormal Trf glycosylation profile and coagulation factors levels. Intestinal lymphangiectasias could also be due to poor compliance and explain the episodes of diarrhea in some patients (D,E). All clinical and biological symptoms as well as Trf glycosylation profile improved with a good compliance as previously described.[2,21] The most severe complication was pulmonary embolism upon D-mannose treatment interruption (patient C). No adverse effect was observed except one patient (E) who developed mild renal failure after 13 years of treatment. It is not clear if it is due to treatment or to the natural course of the disease.

Toxic effects of D-mannose supplementation have been reported in the literature. Neurological complications attributed to intracellular adenosine triphosphate (ATP) deprivation were described after intravenous D-mannose administration.[22] The toxicity of high doses of D-mannose have been showed to be toxic through the accumulation of mannose-6-phosphate leading to ATP depletion[23] and inhibition of glycolysis.[22,24] In mouse models, the results are contradictory regarding the embryonic lethality of D-mannose[16,24,25] which is why we decided to stop D-mannose during our patient's pregnancy. Normal pregnancy without D-mannose treatment in MPI-CDG patients has been previously described.[2,8]

We reviewed serum mannose dosages (peak and residual) and WB Trf profiles over time. We did not identify any correlation between D-mannose treatment (dose, frequency and type of administration) and severity of liver disease or WB Trf profile. We did observe a clear correlation between a normal WB Trf profile and good compliance, indicating that WB Trf profile normalization rather reflects a chronic exposure (ie, good compliance) to treatment. Factor XI, anti-thrombin III, proteins C and S levels correlated with compliance to treatment.

The frequency of poor compliance underlines the need to develop more convenient galenic forms of D-mannose that favor more reliable intake. To monitor compliance, we propose to perform hemostasis and Trf glycosylation profile regularly during the follow-up rather than serum mannose level, which finally did not prove useful in our experience.

5 | CONCLUSION

We describe the largest series of MPI-CDG patients treated with D-mannose up to 20 years, with a median treatment duration of 14 years and 9 months. All treated patients are alive with improved clinical phenotype. We confirm that mannose should be pursued long term to prevent the risk of thrombosis or recurrence of digestive symptoms. Compliance should be monitored by hemostasis and Trf glycosylation profile, and the development of a sustained released form of D-mannose would aid in compliance. Treatment may improve lobular fibrosis but portal fibrosis will persist. The main complications are related to portal hypertension and its consequences. Our study emphasizes the necessity to follow up in adulthood the following: (i) liver disease and the progression toward portal hypertension, (ii) venous thrombosis (in particular in women), (iii) renal function, and (iv) compliance to D-mannose treatment.

ACKNOWLEDGMENT

We thank the patients and the families for their collaboration. This work was supported by patient's associations (Nos Anges, OPPH, Hyperinsulinisme, Noa Luû). We are also grateful to the doctors of the several primary centers that have in charge patients: Dr Peralta, Dr Kretz, Dr Bardoux-Jacquet, and Dr Dabadie. This work was supported by grant ANR-15RAR3-0004-06 under the frame of E-RARE-3, the ERA-Net for Research on Rare Diseases; it was also supported by the European Union's Horizon 2020 research and innovation program under the ERA-NET cofound action N° 643578.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Girard M, Douillard C, Debray D, et al. Long term outcome of MPI-CDG patients on D-mannose therapy. *J Inherit Metab Dis*. 2020;1–10. https://doi.org/10.1002/jimd.12289