ORIGINAL ARTICLE

Clinical, laboratory and molecular findings and long-term follow-up data in 96 French patients with PMM2-CDG (phosphomannomutase 2-congenital disorder of glycosylation) and review of the literature

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ABSTRACT

Background Phosphomannomutase 2-congenital disorder of glycosylation (PMM2-CDG) is a multisystem inborn error of metabolism.

Objectives To better characterise the natural history of PMM2-CDG.

Methods Medical charts of 96 patients with PMM2-CDG (86 families, 41 males, 55 females) were retrospectively reviewed. Data on clinical, laboratory and molecular parameters at diagnosis were analysed. Follow-up data at last examination were reported for 25 patients.

Results The patients were born between 1963 and 2011. Diagnosis of PMM2-CDG was made at a mean (SD) age of 6.8 (8.5) years. The presenting signs were mostly neurological (hypotonia, intellectual disability, cerebellar syndrome) and observed in almost all the patients. A total of 38 patients (14 males, 24 females) exhibited, in addition to neurological signs, visceral features including at least one of these: feeding difficulty requiring a nutritional support (n=23), cardiac features (n=20; pericarditis: 14, cardiac malformation: 9, cardiomyopathy: 2), hepato-gastrointestinal features (n=12; chronic diarrhoea: 7, protein-losing enteropathy: 1, ascites: 3, liver failure: 1, portal hypertension: 1), kidney features (n=4; nephrotic syndrome: 2, tubulopathy: 2) and *hydrops fetalis* (n=1). Twelve patients died at a mean age of 3.8 years (especially from pericarditis and other cardiac issues). Laboratory abnormalities mostly included elevated transaminases and abnormal coagulation parameters. High thyreostimulin levels, hypocholesterolemia, hypoalbuminemia and elevated transaminases were associated with the visceral phenotype. Besides the common Arg141His *PMM2* variant harboured by half of the patients, 45 different variants were observed.

Conclusions PMM2-CDG clinical phenotype is heterogeneous in terms of clinical course, with no clear division between neurological and visceral presentations.

INTRODUCTION

Congenital disorders of glycosylation (CDG) are a group of genetic defects in the synthesis and attachment of glycoprotein and glycolipid glycans including the N-glycosylation and O-glycosylation defects that are, respectively characterised by abnormal N-glycosylation and O-glycosylation of a number of serum glycoproteins.[1] Phosphomannomutase 2-CDG (PMM2-CDG) is the most frequent form of N-glycosylation defects related to pathogenic variants in *PMM2*.[2] PMM2-CDG is characterised by a wide variety of clinical presentations based on the affected organs.[3] The phenotype varies from very severe to mild. Neurological symptoms are frequent including cerebellar ataxia, intellectual disability (ID), retinitis pigmentosa and peripheral neuropathy.[4–6] However, mild neurological presentations are also reported.[7–9] Importantly, well-preserved neurodevelopment or normal IQ should not rule out PMM2-CDG.[9] This diagnosis has also to be considered in children with symptoms such as failure to thrive, pericarditis[4] or conotruncal heart defects[10] or in patients with laboratory abnormalities including elevated serum transaminases, abnormal clotting factors, hypoglycaemia and hypothyroidism. Finally, PMM2-CDG has been described in children with mild clinical findings and any kind of unexplained multisystem disorder, and a few reported cases were even asymptomatic.[11]

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This heterogeneous phenotype may also mimic other inborn errors of metabolism, particularly mitochondrial diseases.[12] Screening is based on abnormal glycosylation of serum N-glycoproteins, especially transferrin. Definitive diagnosis is to be confirmed by molecular analysis (*PMM2* sequencing). However, late diagnosis can also occur due to uncertain results of these screening tests such as mild transferrin abnormalities or even negative screening.[13 14]

Initial clinical descriptions of patients with PMM2-CDG, mostly reported in the late 1990s to early 2000s included only a small number of patients. In 2001, we reported the clinical and laboratory findings of 26 patients with PMM2-CDG and proposed a classification into an isolated neurological form and a neuro-visceral form strongly related with clinical severity. However, since 2001, most publications showed more complex ties between neurological and visceral symptoms, notably patients with mild ID despite significant multiorgan abnormalities.[15] Moreover, numerous patients with PMM2-CDG have been described surviving into adulthood.[6 16] Some of them are already in their 60s and exhibit a relatively milder phenotype than anticipated, based on the first early lethality descriptions in childhood.[16]

To better delineate the range of clinical presentation and long-term outcomes of patients with PMM2-CDG, we analysed clinical, laboratory, molecular and follow-up data of a large cohort of patients retrospectively reviewed over two decades.

METHODS

Population

Inclusion criteria of patients with PMM2-CDG diagnosed in France were an abnormal CDG-I glycosylation pattern of serum glycoproteins identified by western blotting assay,[17] a decreased cytosolic PMM activity in either blood mononucleated leukocytes or cultured skin fibroblasts and the presence of variants in *PMM2*. Pathogenic variants in the *PMM2* gene were identified in the affected individual as previously reported,[4] and Sanger sequencing showed that the parents were heterozygous for either variant. All of them were either known pathogenic variants (reported previously or proved to be pathogenic by functional studies) or variants predicted as pathogenic by several prediction programmes.[18] Moreover, clinical data for diagnosed patients were to be available for inclusion of the patients in this study. Ten patients for whom clinical data were missing were excluded. Adult patients described in a previous series were included.[16]

Data collection

The medical data were retrospectively obtained in 2012–2013 from medical reports including physical examination, nutritional parameters, organs and neuropsychological testing and drug treatments. Each clinical data and laboratory data at diagnosis and at the last consultation during the follow-up were collected.

Besides antenatal signs, clinical symptoms included neurological and visceral features. Visceral signs were feeding difficulty requiring a nutritional support (enteral tube, gastrostomy), cardiac features (pericarditis, cardiomyopathy, cardiac malformation), hepato-gastrointestinal (GI) symptoms (chronic diarrhoea, protein-losing enteropathy, ascites, liver failure, portal hypertension), kidney (nephrotic syndrome, proximal tubulopathy) and *hydrops fetalis*. Patients with at least one of these visceral features were grouped into the 'visceral' group. Patients with none of these visceral signs were grouped in the 'non-visceral group'. Hepatomegaly, fibrosis and cirrhosis could not be included among the visceral signs as not all patients required liver biopsy/liver ultrasounds. Also, vomiting was not included as it could be due to gastro-oesophageal reflux. Sensorineural issues (eg, deafness and retinal finding) and endocrine manifestations were separately reported.

Neurological signs included hypotonia, cerebellar ataxia, psychomotor retardation and ID (IQ<70). Neurocognitive evaluation using standardised psychological tests according to patient age (developmental quotient by Brunet-Lézine test, IQ by the WPPSI-R (Wechsler Preschool and Primary Scale of Intelligence); WISC-III and WISC-IV (Wechsler Intelligence Scale for Children)) was performed. The other criteria for neurological outcome were patient's schooling classified as normal with normal academic achievement for age, in remedial classes or institutional education when patient had minor handicap and absence of school in the presence of severe ID. For adults, socio-professional integration and autonomy were investigated.

Statistical analysis

Descriptive statistics such as range (minimum–maximum, min–max), median with 25th and 75th percentiles, mean with SD and frequency were used to summarise patient characteristics. The relationship between phenotypes and genotypes were assessed, and the intrafamilial heterogeneity was studied. Trends over time of clinical signs were also examined. Fisher's exact and χ^2 tests were performed to examine the relationship between categorical variables. Continuous levels across categories were compared using Kruskal-Wallis test. Laboratory abnormalities during the follow-up were studied using Wilcoxon signed-rank tests.

Statistical analyses were performed with STATA (release 11; Stata).

RESULTS

Study population

Table 1 shows the main characteristics of the study population. Ninety-six French patients (from 86 unrelated families, 41 boys

Table 1 Characteristics of the study population: 96 patients with PMM2-CDG

Characteristic	n (%) or mean (SD)
Sex (n=96)	
Female	55 (57.3)
Male	41 (42.7)
Birth parameters	
Weight (g) (n=70)	3076.7 (517.4)
Height (cm) (n=63)	48.6 (2.8)
Head circumference (cm) (n=54)	34.6 (3.0)
Diagnostic age (years) (n=92)	6.8 (8.5)
Age at last examination (years) (n=94)	12.0 (10.0)
Living place (n=75)	
Family house	44 (58.7)
Institution	7 (9.3)
Family house and institution	23 (30.7)
Other	1 (1.3)
Schooling (n=75)	
Normal	13 (17.3)
Adapted	34 (45.4)
Not attending school	19 (25.3)
Not applicable (too young)	9 (12.0)
Death (n=78)	12 (15.4)

Values are frequencies (n, %) or mean (SD).
PMM2-CDG, phosphomannomutase 2-congenital disorder of glycosylation.

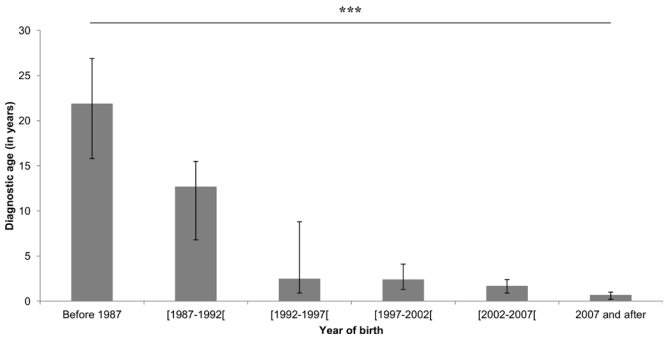

Figure 1 Diagnostic age by year of birth of French patients with PMM2-CDG. Bars present medians of diagnostic age, and errors bars the interquartile ranges (25th and 75 percentiles) ***p<0.001 (p value from Kruskal-Wallis test). PMM2-CDG, phosphomannomutase 2-congenital disorder of glycosylation.

and 55 girls, born between 1963 and 2011) were included in the study. At the last visit, they were 12.0 (10.0) years old on average (min–max: 1 month–49 years), and 28 patients (16 girls, 12 boys) were older than 15 years.

Diagnosis of PMM2-CDG was made at a mean (SD) age of 6.8 (8.5) years (min–max: 1 day–38 years). Data on presenting symptoms were available for 71 patients. Neurological symptoms were the most common presenting symptoms (reported in 60 patients). Specifically, hypotonia was reported as a presenting sign in 25 patients, developmental delay in 17, cerebellar syndrome in 11 and unspecified neurological signs in 7 patients. As expected, an earlier age at diagnosis was observed after 1997 (figure 1).

About one-third of the patients (36%, n=32/88) were taken care of by a physician from a rehabilitation centre, 64% (n=56/88) from a reference centre for inherited metabolic diseases (37 of them also from an academic hospital) and none by their family doctor alone. A majority of patients (n=59) were treated with medication.

Patients needed support in everyday life activities and lived in their families, in addition to rehabilitation centres for one-third of them (34%, n=23/67). While 13 school-aged patients were enrolled in a normal school (mean (SD) age at the last visit: 18.1 (15.7) years), 34 school-aged patients required adapted school training (13.0 (8.0) years) and 19 were out-of-school (13.3 (8.9) years). Only one adult patient (24.9 years, genotype: Cys241Ser/Phe157Ser) had a work without adaptation, three with adaptation (28.8 (2.3) years, genotypes: Cys241Ser/Arg141His, Thr18Ser/c.255+2T-C and Phe113Leu/Arg141His) and five were unemployed (33.5 (13.1) years, Cys9Tyr/Arg141His, Cys9Tyr/Arg141His, Thr237Arg/Arg162Trp, Cys241Ser/Phe157Ser, Cys241S/Arg141His). Based on the presence of visceral signs, patients were grouped into 'visceral' group and 'non-visceral' group (table 2).

Detailed clinical presentation

Perinatal parameters and growth

Birth parameters were mostly in the normal range (table 1), although pregnancy was reported as abnormal in 19 of 59 cases. Antenatal manifestations, such as conotruncal cardiopathy (n=4) and inverted nipples (n=3), were recorded.

Failure to thrive was observed in 42 of 80 patients and more frequently in patients from the visceral group (table 2).

Visceral signs

A total of 38 patients (14 boys, 24 girls) presented at least one visceral symptom. The visceral signs occurred early in life, before 2 years of age in 27 patients. Feeding difficulties (n=23 including enteral tube, gastrostomy feeding), cardiovascular (n=20 including pericarditis, cardiac malformations, cardiomyopathy) and GI issues (n=12 including enteropathy, ascites, liver failure, portal hypertension) were the most frequent signs. The distribution of all visceral signs used in the definition of the visceral group is shown in figure 2. A total of 10 patients exhibited both feeding difficulties and cardiac issues.

Additional non-specific signs

Recurrent *vomiting* and *hepatomegaly* were reported in 16 and 19 patients, respectively. Liver biopsy revealed steatosis, fibrosis and cirrhosis: steatosis was observed in nine patients including six from the visceral group, fibrosis and cirrhosis in six patients including four from the visceral group.

Morphological signs and skin abnormalities

Dysmorphic symptoms were noted in 55 patients, including prominent forehead, large ears and up-slanting palpebral fissures. *Skin abnormalities*, mostly orange peel skin, inverted nipples and fat pads, were noted in early infancy in 61 patients.

Endocrine involvement (other than thyroid)

Ovarian dysplasia was observed in 8 females aged 17.4 (6.6) years on average, while ovarian insufficiency was observed in 17 girls (17.1 (4.9) years). Spontaneous menstruation was only documented in one female patient. Hormonal substitution therapy with natural oestrogen estradiol was used in 14 female patients. Micropenis was documented in two boys younger than 2 years of age.

Sensorineural involvement

Sensorineural deafness was noted in four patients (information available for 25 patients), all in the visceral group. The average age at diagnosis of sensorineural deafness was 9.4 (12.2) months (min–max: 1–27.3 months). *Ophthalmological abnormalities* were frequently reported in 86 patients aged 6.6 (7.2) years on average, including strabismus (n=80), retinitis pigmentosa (n=20), nystagmus (n=15), astigmatism (n=14) and myopia (n=14). Only one patient had normal retinal findings at the first visit (4 months of age) and exhibited a retinopathy at the second visit at the age of 14.7 years. Rare findings were optic atrophy (n=2) and coloboma (n=1).

Skeletal involvement

Skeletal abnormalities were reported in 68 patients, not only kyphosis and scoliosis (n=40) but also osteopenia (n=16), joint hyperlaxity (n=17) and thorax deformation (n=17), at 7.1 (6.7) years on average. More than half of the patients exhibited skeletal abnormality before the age of 6 years (median age: 4.8 years).

Neurological involvement

Data on motor involvement were available for 92 of the 96 patients. All of these 92 patients had motor involvement: axial hypotonia in 65 patients and cerebellar ataxia in 76 patients. Cognitive issues were reported in 89 patients. ID was observed in 36 patients (data available for 39 patients). Normal language was reported in 10 patients, while 14 were able to read. Twelve patients were able to walk before the age of 2 years, and 22

Table 2 Clinical and laboratory findings in all patients and by group (visceral vs non-visceral)*

Characteristic (n=number of patients with available data)	All patients n=96 n (%)	Visceral group n=38 n (%)	Non-visceral group n=58 n (%)
Failure to thrive (n=80)†	42 (52.5)	24 (75.0)	18 (37.5)
Dysmorphic symptoms (n=62)	55 (88.7)	22 (95.7)	33 (84.6)
Motor involvement (n=92)	92 (100)	36 (100)	56 (100)
Axial hypotonia (n=76)	65 (85.5)	32 (94.1)	33 (78.6)
Lower limb hypotonia (n=47)	35 (74.5)	16 (80.0)	19 (70.4)
Ataxia (n=60)	58 (96.7)	18 (100)	40 (95.2)
Cerebellar syndrome (n=78)	76 (97.4)	25 (96.2)	51 (98.1)
Epilepsy (n=67)	32 (47.8)	16 (59.3)	16 (40.0)
Stroke-like episodes (n=24)	12 (50.0)	6 (54.6)	6 (46.2)
Demyelinating neuropathy (n=12)	7 (58.3)	4 (66.7)	3 (42.9)
Axonal neuropathy (n=11)	3 (27.3)	1 (20.0)	2 (33.3)
Cognitive issues (n=96)†	89 (92.7)	32 (84.2)	57 (98.3)
Normal development of speech and language skills (n=70)	10 (14.3)	2 (9.1)	8 (16.7)
Counting (n=32)†	12 (37.5)	1 (8.3)	11 (55.0)
Reading (n=39)†	14 (35.9)	2 (14.3)	12 (48.0)
Walking autonomously before 2 years (n=75)	12 (16.0)	2 (8.0)	10 (20.0)
Walking autonomously before 10 years (n=49)†	22 (44.9)	4 (22.2)	18 (58.1)
Hearing (n=27)	7 (25.9)	5 (50.0)	2 (11.8)
Sensorineural deafness (n=25)†	4 (16.0)	4 (44.4)	0 (0)
Eye involvement (n=88)	86 (97.7)	31 (96.9)	55 (98.2)
Strabismus (n=84)	80 (95.2)	28 (93.3)	52 (96.3)
Retinitis pigmentosa (n=41)	20 (48.8)	9 (52.9)	11 (45.8)
Nystagmus (n=17)	15 (88.2)	5 (83.3)	10 (90.9)
Astigmatism (n=21)	14 (66.7)	5 (62.5)	9 (69.2)
Myopia (n=22)	14 (63.6)	3 (42.9)	11 (73.3)
Optic atrophy (n=21)	2 (9.5)	1 (9.1)	1 (10.0)
Coloboma (n=27)	1 (3.7)	0 (0)	1 (6.3)
Bone involvement (n=78)	68 (87.2)	28 (93.3)	40 (83.3)
Kyphosis and scoliosis (n=57)	40 (70.2)	19 (82.6)	21 (61.8)
Osteopenia (n=29)	16 (55.2)	9 (75.0)	7 (41.2)
Articular hyperlaxity (n=27)	17 (63.0)	4 (66.7)	13 (61.9)
Thorax deformation (n=34)	17 (50.0)	10 (76.9)	7 (33.3)
Laboratory results‡			
Albumin levels<35 g/L (n=42)†	12 (28.6)	8 (40.0)	4 (18.2)
Cholesterol levels<4 mmol/L (n=46)†	34 (73.9)	16 (88.9)	18 (64.3)
Alanine aminotransferase>50 U/L (n=58)†	25 (43.1)	16 (66.7)	9 (26.5)
Prothrombin time>80% (n=61)	47 (77.1)	21 (75.0)	26 (78.8)
Antithrombin<50% (n=39)†	13 (33.3)	8 (61.5)	5 (19.2)
Protein C<50% (n=44)†	14 (31.8)	9 (64.3)	5 (16.7)
Protein S<50% (n=37)	2 (5.4)	1 (10.0)	1 (3.7)
Thyreostimulin>5 mU/L (n=45)†	4 (8.9)	4 (25.0)	0

The number of observations per characteristic is reported next to the item considered (first column). All other values are frequencies (%). Between brackets are the percentages of patients out of the number of available data (second, third and fourth columns). For instance, data on failure to thrive were available for 80 patients, and 42 out of 80 patients had a failure to thrive (ie, 52.5%).

*Detailed visceral signs are reported in figure 2.

†p<0.05 (p value from χ^2 test or Fisher's exact test to test for a statistically significant relation between the item of interest and visceral/non-visceral group).

‡Laboratory parameters recorded at diagnostic visit.

n, number of observations.

before the age of 10 years. Sixteen patients were autonomous for dressing and holding a pencil.

Brain MRI abnormalities were observed in all patients for whom a brain MRI had been performed (n=80). These patients aged from 14 days to 31 years and exhibited: cerebellar atrophy (n=77), cerebral atrophy (n=8) and rarely white matter hyperintensities (n=4). Electromyogram data were reported in 23 patients (mean age: 5.2 (6.1) years; min–max: 27 days–24 years), abnormalities were observed in 16 patients, demyelinating neuropathy in 7 patients, axonal neuropathy in 3 and other abnormalities in 6 patients.

Epilepsy was noted in 32 patients and tended to be more frequent in patients with visceral signs (χ^2 test, p=0.122). Epilepsy occurred at a mean age of 2.7 (3.3) years. Twenty-six patients were on anticonvulsant therapy. Epilepsy was deemed as well controlled by treatment in 16 patients (available information

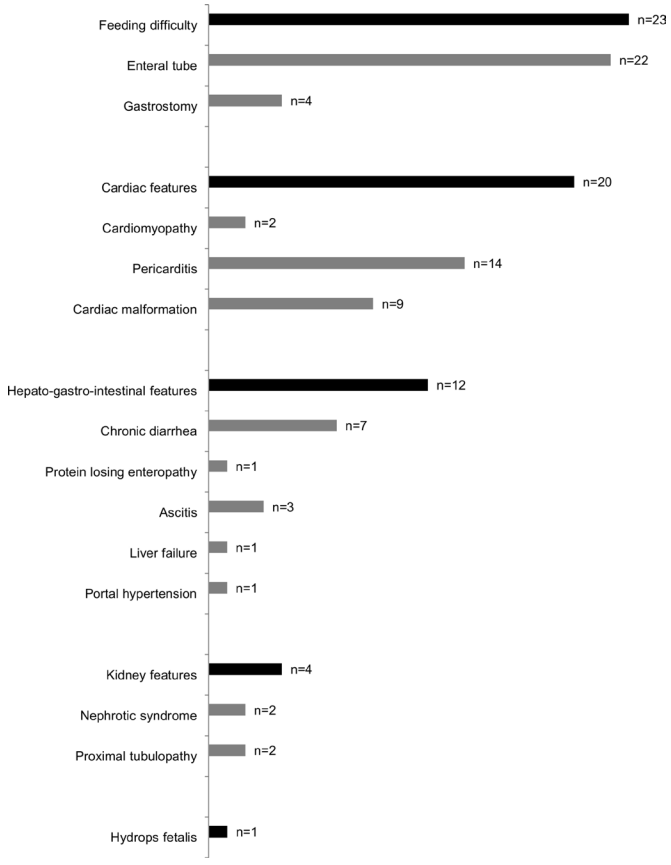

Figure 2 Distribution of the visceral signs used in the definition of the visceral group.

for 19 patients). No patient exhibited more than one seizure per day. One patient exhibited between one seizure per day and one seizure per month. Thirteen patients exhibited less than one seizure per month.

Vascular-related events were noted in 19 patients including 12 stroke-like episodes, 4 cerebral thrombosis and 2 cerebral haemorrhages and a peripheral venous thrombosis in 2 cases (1 patient had both stroke-like episodes and cerebral thrombosis). These episodes were reported at 8.8 (6.6) years on average (min–max: 14 days–22 years). A stroke-like episode was defined as an acute, sudden and usually transient neurological deficit clinically similar to a cerebrovascular accident but with different neuroimaging characteristics. Brain MRI in stroke-like episode usually demonstrates a localised zone with restricted diffusion (cytotoxic oedema) that does not correspond to any artery territory. Stroke-like episodes that occurred during fever were significantly linked to seizures as 9 of 12 patients with stroke-like episodes had a concomitant seizure (Fisher's exact test, p=0.009). Patients with vascular-related events had or tended to have lower diagnosis levels of factor XI (median levels of factor XI: 15.5% vs 58.0% in patients without vascular-related events, Kruskal-Wallis test, p=0.002), protein C (35.0% vs 71.5%, p=0.021), protein S (64.0% vs 79.5%, p=0.122) and antithrombin (32.0% vs 65.0%, p=0.074). There was no specific genotype associated with vascular-related events.

Medical records reported information about *rehabilitation* for 62 patients. Eleven patients did not require any rehabilitation and the others needed one (n=18) to more than three types of rehabilitation (n=9): physical therapy, speech therapy, occupational therapy.

Intrafamilial presentation

Nine families comprised more than one affected individual: seven with two siblings, one with three siblings and one family with the aunt and the niece.

Intrafamilial clinical homogeneity was generally observed. However, in two non-visceral families, cerebellar ataxia and epilepsy were, respectively, described in only one out of the siblings. In one family with two affected siblings, sensorineural deafness was noted in only one. The severity of visceral signs could also vary within a family.

Follow-up

Data were collected twice (diagnosis time and last medical visit) in 25 patients (14 patients from the visceral group).

Neurological signs were definitively non-progressive. Conversely, visceral signs could increase or decrease in number or intensity. Nine out of 14 patients with early visceral features continued having them, while these features disappeared in 4 out of 14 patients and appeared in 1 out of 14 patients.

Only one patient changed from no schooling to special education. An increase in the number of types of rehabilitation was reported in three patients.

Laboratory abnormalities

At diagnosis

Laboratory abnormalities were reported but with a great variability in the type and severity. Using data at diagnostic visit, the main results were high liver enzymes in 43.1% of the patients (alanine aminotransferase [ALAT]>50 U/L), as well as abnormal coagulation parameters such as low antithrombin (33.3%, antithrombin<50%), protein C (31.8%, protein C<50%) and protein S levels (5.4%, protein S<50%). Prothrombin time was normal (>80%) in 77.1% of the patients. Mean (SD) factor IX (available in 37 patients), factor XI (n=44) and factor XII (n=24) were 76.4 (24.0)%, 50.2 (32.4)% and 96.5 (29.7)%, respectively. Factors IX, XI and XII were in the normal range (>70%) for 54.1%, 29.5% and 83.3% of patients, respectively. Mean (SD) cholesterol value (n=46) was 3.4 (1.0) mmol/L, with 73.9% patients for whom values were <4 mmol/L (and 21.7% of patients exhibiting cholesterol levels in the normal range: 4–5.2 mmol/L). Hypoalbuminemia, high liver enzymes, low antithrombin and protein C levels and hypocholesterolemia were mainly observed in visceral patients (table 2).

Thyroid-related hormone blood levels were available for 45 patients. Among these, four patients (8.9%), all from the visceral group, had high thyreostimulin (TSH) levels (>5 mU/L, normal values 0.6–4 mU/L) with free thyroxine (T4) blood levels ranging from 7.0 to 14 pmol/L (normal values 9–15 pmol/L). Unfortunately, detailed information about replacement therapy with levothyroxine was unavailable at the time of thyroid-related hormone blood measurement with some patients possibly treated and others not.

During follow-up

For the patients with measurements at two different time points, no difference in laboratory parameters levels was observed, except for ALAT, factor XI and TSH. ALAT and TSH decreased over time (median (P25–P75) of ALAT: 67 (41–107) vs 40 (24–51) U/L, Wilcoxon signed-rank test, p=0.013, and TSH: 3.5 (2.5–3.8) vs 1.9 (1.2–2.9) mU/L, p=0.047). Factor XI increased over time (9.5% (7.0%–29.0)% vs 25 (22–63)%, p=0.028).

A child who had normal laboratory results in his routine screening exhibited a stroke-like episode at the time of a fever: at

that time his laboratory results were mildly altered with increased ALAT and decreased antithrombin (53%) and protein C levels (50%). Factors XI and IX were 70% and 90%, respectively.

Molecular findings

A great genetic heterogeneity was observed: 46 different *PMM2* pathogenic variants were identified including a majority of heterozygous (only two homozygous variants: Asp65Tyr and Leu32Arg). These variants were mostly missense (93%) and scattered throughout the gene. As expected, the Arg141His variant was the most frequent (present in half of the families), and the second frequent variant was Glu139Lys, but to a far lesser extent (in nine families only). The remaining variants were therefore rarely identified, hampering genotype–phenotype correlation.

Relationship between visceral and non-visceral groups and genotype was seldom observed. Patients with genotype Arg141His/Val129Met (three out of three patients), Arg141His/Cys9Tyr (two out of two patients) and Arg141His/Val231Met (three out of four patients) could be affiliated to the visceral group, whereas patients with genotype Arg141His/Ala108Val (four out of four patients), Arg141His/Gly214Ser (two out of two patients) and Arg141His/Cys241Ser (three out of four patients) could be affiliated to the non-visceral group.

Death

The death of 12 patients was reported, at a mean age of 3.8 (7.7) years (min–max: 1 month–25 years), from various visceral causes: pericarditis and other cardiac abnormalities (n=4), infection (n=2), liver failure (n=1), haemorrhage (n=1) and not reported (n=4). The number of deaths was significantly related to the number of visceral signs: two deaths in non-visceral group from unclear reasons, four deaths in visceral group with one sign and six deaths in visceral group with ≥2 signs (Fisher's exact test, p=0.001). All patients with genotype Val231Met/Arg141His died (n=4), as well as patients with the following genotypes: Asp65Tyr/Arg141His (n=1), Asp65Tyr/Phe157Ser (n=1), Asp65Tyr/c.179-25A>G (branch site) (n=1), Val231Met/Phe207Ser (n=1). Two out of six patients with genotype Phe119Leu/Arg141His and two out of seven patients with genotype Glu139Lys/Arg141His also died.

DISCUSSION

This study presents the clinical and laboratory abnormalities of 96 French patients with PMM2-CDG covering different ages, from birth to adulthood. While most of the patients presented neurological signs, a total of 38 patients exhibited visceral features. Our data re-emphasise the variability of neurological and visceral symptoms and the non-progressive characteristics of neurological symptoms. Hypotonia and cerebellar hypoplasia are pathognomonic signs present in most of the patients.

CDG screening was performed in the setting of an unspecific clinical presentation including mostly neurological symptoms notably cerebellar ataxia and visceral signs (pericarditis and coagulation anomalies).

In 2001, 26 patients with PMM2-CDG were categorised into an isolated neurological form and a neurovisceral form more severe and lethal than the former one.[4] The data presented herein are in line with our previous description. Regardless, the two distinct presentations do more or less coexist, but they do not predict the severity of the symptoms themselves, as well as the vital prognosis. The extra-neurological manifestations, more frequent in girls, occurred early in life. Death (12 patients with different age) occurred mostly in patients with the visceral form and an increasing number of visceral signs, especially pericarditis and cardiac issues, as reported.[3] Interestingly, since the initial description of PMM2-CDG patients, we do see a higher rate of long-term survival in patients,[19] even with multiple visceral features. This is probably due to improvement of care with supportive therapies, nutrition, early use of antibiotics and avoidance of elective surgeries that are determining outcome factors.[19 20]

Symptoms of PMM2-CDG considered as relatively specific, such as dysmorphic features, inverted nipples and abnormal fat pads,[3] were occasionally absent in both forms, but present in 33 out of 58 non-visceral patients and in 22 out of 38 visceral cases.

Among the commonly described neurological symptoms, strabismus, psychomotor retardation and cerebellar hypoplasia occurred early on, while peripheral neuropathy and retinitis pigmentosa were frequently observed in the course of the second year of life. The main neurological symptoms remained cerebellar dysfunction, resulting from a combination of cerebellar hypoplasia and atrophy, variably reported as cerebellar hypotrophy, olivopontocerebellar hypoplasia or cerebellar atrophy.[21–23] Cerebellar ataxia was clinically non-progressive as only neuroimaging picture evolved, suggesting a developmental disease. Indeed, cerebellar hypoplasia is an antenatal-onset symptom, occurring early, when the external granule layer—which appears at the end of the embryonic period and persists up to 2 years after birth—is still playing an active germinal role and the development of the cerebellum is still incomplete.[22 23] A link between the severity of cerebellar symptoms and neuroimaging findings has, however, been proposed in PMM2-CDG.[24] Several autopsy findings show widespread cerebellar atrophy, with the anterior lobe vermis more severely affected.[25] In these studies, microscopic studies show complete loss of Purkinje cells and subtotal loss of granule cells throughout the cerebellar cortex. Pontine nuclei and the inferior olives have various degrees of neuronal depletion and severe gliosis. The cerebellar hemispheres are less involved.

Retinitis pigmentosa, observed in 20 of our patients, is an ophthalmological symptom very commonly reported in adult PMM2-CDG[26] identified by electroretinogram more than by fundus oculi examination, which is less sensitive. This visual alteration may have rarely a major impact on quality of life, as in our cohort visual acuity was preserved. However, most of our patients were young, while tapetoretinal degeneration is slowly progressive in the preschool/school age.[26] Retinal alteration in PMM2-CDG could be related with abnormal protein composition of photoreceptors.[26]

Considering peripheral nervous system, mild peripheral neuropathy is also a frequently reported symptom, with prominent involvement of motor nerves.[27] Electrophysiology was not performed in all patients.

Stroke-like episodes are frequently reported (40%–55% of cases).[28–30] Similarly, epileptic seizures are not uncommon in patients with PMM2-CDG and are often observed in association with stroke-like episodes.[31] Epilepsy was described in one-third of our patients, occurring early in life, while an acute cerebral event occurred in one-sixth of our patients including 12 patients with stroke-like episodes. Interestingly and as reported, there was a significant relationship between stroke-like events and seizures in our patients. Therefore, from our experience, in the setting of an acute neurological episode, it is key to consider both seizure and stroke-like episode. These acute episodes were triggered by infection, as previously described.[29 31] The revealing symptoms could be irritability, mental status changes and hemiparesis and sometimes seizures clinically visible, and their

duration varied, with recovery ranging from days to weeks.[7 21] Cytotoxic oedema followed by focal necrosis and atrophy in the left temporo-occipital watershed area are described.[22 30] Unbalance between procoagulant and anticoagulant factors has been proposed as a possible explanation for these stroke-like events because both procoagulant (factor XI) and anticoagulant (protein C, antithrombin) proteins are reported to be deficient in patients with PMM2-CDG,[28 29 32] as well as in our cohort. Normalisation of factors IX and XI activity might occur during maturation, paralleling the disappearance of transferrin isoform abnormalities.[19] Moreover, a normal transferrin pattern can become altered during infections, which are a situation at risk for patients with PMM2-CDG. Thrombosis remains also a common life-threatening complication in adults with PMM2-CDG.[33] Platelet hyperaggregability and transient decrease of endogenous anticoagulants during catabolic stress are other arguments for ischaemia.[34] In a former study, we recommended prophylaxis with low-dose aspirin after a first arterial thrombosis or stroke-like episode.[29] Nonetheless, we have been following a patient since 2000 with repeated stroke-like episodes despite low doses of aspirin. Therefore and because patients can suffer from liver cirrhosis with portal hypertension, the use of aspirin prophylaxis for stroke-like episodes should be discussed on a case-by-case basis.

Visceral signs are heterogeneous in the clinical presentation,[3 4] from multiorgan failure to hyperinsulinemia–hypoglycaemia, even mimicking mitochondrial disease (notably one patient with hemiplegic headache, stroke-like episodes, ophtalmoplegia, ataxia and neuropathy in our cohort).[12] Most of the multiorgan manifestations appear in infancy. Nutritional support reflecting failure to thrive and feeding difficulty was the most frequent visceral sign reported followed by cardiac features. Half of the patients who required an enteral nutrition had cardiac involvement. While pericardial effusion is estimated to occur in 30% of patients with PMM2-CDG,[3 35] the frequency of pericarditis, the most frequent cardiac feature, was 15% in our cohort.

Interestingly, sensorineural deafness was only diagnosed in cases with visceral presentation.

Non-immune *hydrops fetalis* (abnormal accumulation of fluid in two or more fetal compartments) has also been described in patients with PMM2-CDG, with skin oedema, ascites, pleural effusion, pericardial effusion, polyhydramnios and hydropic placenta,[20 36 37] leading to prenatal expression of the disease. Hypertrophic obstructive cardiomyopathy in the prenatal and postnatal period was also reported[38] as well as malformative cardiomyopathies such as conotruncal heart defects.[10] Numerous proteins implicated in the neural crest migration and more generally in prenatal development are glycosylated. In our study, abnormal pregnancy was noted in 32% of the analysable cases, and antenatal manifestations including conotruncal cardiomyopathy and inverted nipples were recorded. This higher frequency might be related with the systematic monitoring of pregnancy by second trimester obstetrical ultrasound in France.

Other visceral features include GI, pancreatic and liver involvement. Abnormal intestinal morphology has been described, with inflammatory cells and decrease of height of villi on light microscopic study of the small intestine, associated with abnormal endoplasmic reticulum, and low activity of lipase in duodenal juice.[39] Postnatal failure to thrive is frequent in severe forms,[40] while fetal growth is normal,[41] as confirmed in our results.

Skeletal abnormalities (including osteopenia, thoracic abnormalities, kyphosis and scoliosis) are frequent , although under-recognised,[15 42] with possible severe complications such as myelopathy by severe spinal cord deformity and compression. Interestingly, almost all of our patients presented with skeletal dysplasia, whatever their age. Therefore, physiotherapy and strict clinical follow-up of the spine are crucial. Treatment with oestrogen in relation with hypogonadotrophic hypogonadism to avoid osteoporosis increases the risk of thrombotic events in female patients and must be dosed carefully. Bisphosphonates might be beneficial in patients with recurrent fractures,[42] but none of our patients were treated by bisphosphonates.

Concerning endocrine system, hypogonadism is typical for PMM2-CDG, with premature ovarian failure,[43] found in the great majority of our female teenagers and adults. However, female patients with normal puberty and menstruation have also been described.[6 44] Interestingly, there was no association between ovarian development and ID: one patient with normal puberty had severe mental retardation and walked without support at 11 years only. Conversely, premature menarche was reported in an apparently healthy female with PMM2-CDG.[11] Abnormal thyroid function is also frequent, in approximately 75% of patients with CDG, as a result of abnormal glycosylation of TSH and thyroid-binding globulin.[45]

Skin can also be affected in PMM2-CDG,[46] which is considered as typical when associated with pericarditis or neurological features. Lecca *et al* compared gene expression profiles in healthy control and PMM2-CDG fibroblasts, demonstrating an important induction of genes encoding extracellular matrix proteins, that is, an important production of collagen-I.[46] This pro-fibrotic tissue response could explain the occurrence of orange peel skin.

Limited genotype–phenotype relationships were noted. Surprisingly, severe phenotypes have been reported with a variable residual activity of the mutant proteins.[47] Accordingly, relatively mild variants were found in patients with *hydrops fetalis*, leaving a PMM residual activity of 25%.

In our study, and as previously reported,[4] the most severe phenotype was observed in the 'neurovisceral' patients (both neurological and multivisceral severity) as shown, for instance, by the relationship between deaths and number of visceral signs (see above). However, when attempting to statistically correlate neurological severity scoring to visceral failures, there was only a trend for hypotonia (χ^2 test, $p=0.055$) and epilepsy (χ^2 test, $p=0.122$). Elevated TSH levels, hypocholesterolemia and elevated liver enzymes were associated with a visceral phenotype. Similarly, the clinical phenotype remained comparable among siblings within a given family, but interfamilial neurological impairment variability was observed in families harbouring the same genotypes, as well as in others previously described.[48] This suggests that the severity of the disease is not solely determined by the *PMM2* mutant alleles but that other genes probably modulate the effect of *PMM2* variants, such as the controversial presence of the c.911T>C polymorphism in *ALG6*, which may exacerbate the clinical severity of patients with PMM2-CDG,[49] or a male factor as suggested by our unbalanced sex ratio for visceral features.

We previously suggested that PMM2-CDG in France is underdiagnosed as the estimated incidence is 1/40 000 per year.[8] Here, we confirm that the total number of patients with PMM2-CDG diagnosed in France since 1995, although the disease is well known by paediatricians and the screening largely performed, is less than 150.

In conclusion, we provide data on 96 patients with PMM2-CDG confirming the remarkable heterogeneity in terms of clinical course and outcome, with no clear division between neurological and visceral presentations. Hypotonia and epilepsy tended to be more frequent in visceral patients. Long-term outcomes appear

less severe than initially reported, possibly thanks to a better management of acute episodes such as pericarditis. Such a broad spectrum of CDG manifestations suggests that this condition is still largely underdiagnosed and plead for better recognition and early management. It should be acknowledged that our study has certain limitations. First, the retrospective methodology based on the analysis of patients' files necessarily introduces some bias. Second, in terms of follow-up, we could only retrieve follow-up data for 25 patients out of the 96 included in the study, thereby limiting interpretation regarding outcome. Finally, longitudinal studies of laboratory parameters such as hemostatic factors were not available in this study. Long-term prospective cohort studies will be warranted to analyse such data.

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