A nationwide survey of PMM2-CDG in Italy: high frequency of a mild neurological variant associated with the L32R mutation

Barone R[1], MD, Carrozzi M[2], MD, Parini R[3], MD, Battini R[4], MD, Martinelli D[5], MD, Elia M[6], MD, Spada M[7], MD, Lilliu F[8], MD, Ciana G[9], MD, Burlina A[10], MD, Leuzzi V[11], MD, Leoni M[12] , MD, Sturiale L[13], PhD, Matthijs G[14], PhD, Jaeken J[15], MD, Di Rocco M[12], MD, Garozzo D[13], PhD, Fiumara A[1] , MD.

[1]Pediatric Neurology, University of Catania; [2]Institute for Maternal and Child Health, IRCCS Burlo Garofolo Trieste; [3]Center for Metabolic Diseases MBBM Foundation, San Gerardo Hospital, Monza.[4]Department of Developmental Neuroscience IRCCS Stella Maris, Calambrone Pisa; [5]Unit of Metabolism Department of Pediatrics, Bambino Gesu Children's Hospital Rome; [6]IRCCS Oasi Maria SS., Troina; [7]Department of Pediatrics, University of Torino; [8]Pediatric Clinic, University of Cagliari; [9]Centre for Rare Diseases, University Hospital Udine; [10]Department of Pediatrics University of Padova; [11]Pediatric Neurology – University "La Sapienza" Rome; [12]Unit of Rare Diseases, Department of Pediatrics, Gaslini Institute Genova; [13]Institute for Polymers, Composites and Biomaterials IPCB, CNR Catania; [14]Centre for Human Genetics, KU Leuven; [15]Centre for Metabolic Diseases, University Hospital Gasthuisberg, KU Leuven;

Address correspondence to: Rita Barone, M.D. Pediatric Neurology Policlinico University of Catania Via Santa Sofia, 78 95123 Catania-Italy [rbarone@unict.it] +39 095 3782467

Key Words: CDG **-** Congenital Disorders of Glycosylation - Severe phenotype - Mild neurological variant - PMM2 gene mutation - Transferrin glycosylation - Pharmacological chaperones

ABSTRACT

PMM2-CDG (*PMM2* gene mutations) is the most common congenital disorder of N-glycosylation. We conducted a nationwide survey to characterize the frequency, clinical features, glycosylation and genetic correlates in Italian patients with PMM2-CDG.

Clinical information was obtained through a questionnaire filled in by the referral physicians including demographics, neurological and systemic features, neuroimaging data and genotype. Glycosylation analyses of serum transferrin were complemented by MALDI-Mass Spectrometry (MALDI-MS).

Between 1996 and 2012 data on 37 Italian patients with PMM2-CDG were collected. All the patients with a severe phenotype were unable to walk unaided, 84 % had severe intellectual disability and 81 % microcephaly. Conversely, among 17 mildly affected patients 82 % had independent ambulation, 64 % had borderline to mild intellectual disability and 35 % microcephaly. Epilepsy and stroke-like events did not occur among patients with the mild phenotype. The rate and extent of systemic involvement were more pronounced in severely affected patients. The L32R misfolding mutation of the PMM2 gene occurred in 70 % of the patients with the mild phenotype and was associated with a less severe underglycosylation of serum Tf at MALDI-MS analyses. Despite their different disease severity, all patients had progressive (olivo)ponto-cerebellar atrophy that was the hallmark clinical feature for the diagnosis.

A mild neurological phenotype of PMM2-CDG marked by preserved ambulatory ability and autonomy and associated with L32R mutation is particularly frequent in Italy. PMM2-CDG should be considered in patients with even mild developmental disability and/or unexplained progressive cerebellar atrophy.

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Introduction

Congenital Disorders of Glycosylation (CDG) are genetic diseases due to defects in the synthesis of the glycan moiety of glycoproteins or glycolipids. They are extremely heterogeneous disorders with multisystem to organ-specific manifestations and mostly prominent nervous system involvement. CDG due to defects of protein N-glycosylation and/or O-glycosylation have been described [1-2]. More than 30 gene defects with neurological presentation affect the N-glycosylation pathway resulting from defective synthesis of the dolichol-linked oligosaccharide precursors (CDG-I) or defective remodelling of the protein N-linked glycans (CDG-II) [3-4].
Almost all N-glycosylation defects are detectable by serum transferrin (Tf) glycosylation analyses using isoelectrofocusing (IEF) and immunofixation or via different methods as capillary zone electrophoresis, HPLC and mass spectrometry measurement of underglycosylated Tf glycoforms [5]. In normal serum, Tf glycoforms are represented by tetrasialotransferrin and small amounts of mono-, di-, tri-, penta-, and hexasialotransferrins. In CDG-I disialo- and asialo- are increased while tetrasialotransferrin is decreased (type 1 pattern); in CDG-II an additional increase of mono- and trisialotransferrin is observed (type 2 pattern) [6].
PMM2-CDG (*PMM2* gene mutations) is the most common genetic disorder of protein N-glycosylation. PMM2 enzyme acts in the synthesis of GDP-mannose required in the early steps of N-glycan synthesis. In PMM2-CDG clinical variability is associated with age and disease severity and reflects multifactor determinants [7-9]. Global developmental disability and cerebellar atrophy are hallmark clinical features. In the most severe end of the spectrum, PMM2-CDG behaves as a multisystem disorder presenting with stunted growth, gastrointestinal symptoms, liver and kidney dysfunction, pericardial effusions, coagulation defects, leading to 20 % rate of mortality due to multi-organ failure in infancy [10]. The neurological phenotype comprises strabismus and other abnormal eye movements, ataxia and hyporeflexia. After infancy, neurological symptoms include retinitis pigmentosa, often stroke-like episodes and less frequently epilepsy [4,11].
We performed a nationwide survey with the aim to characterize clinical data, glycosylation and genetic correlates in Italian patients with PMM2-CDG.

Methods

Between 1996 and 2012 we collected data on 37 Italian patients from 29 unrelated families with proven *PMM2* gene mutations. Patients were followed at our centre (n: 15) and in ten other referral centres for neurometabolic diseases spread over the country. Clinical information was obtained by a questionnaire filled in by the referring physicians including demographics, neurological and systemic features with neuroimaging data and genotype. Laboratory diagnosis was performed by characterization of serum Tf isoforms by IEF or HPLC analysis. In fifteen patients, glycosylation analyses were complemented by Maldi- Mass Spectrometry (Maldi-MS) using an ad-hoc developed protocol [12]. PMM2 gene analysis was performed by direct sequencing [13]. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983. Informed consent was obtained from parents or guardians.

Results

A total of 37 patients with PMM2-CDG (25 males and 12 females) ranging in age from 2 to 67 years were included in the study. On the basis of clinical features, PMM2 genotype and glycosylation data, severe and mild phenotypes can be distinguished. Clinical and laboratory data of each patient at the time of this study are summarized in tables 1 and 2. Clinical reports on patients 1-4, 7-8, 21-24, and 27 had been previously published [14-17]. For these patients, follow-up data were included in the present study.

Severe Phenotype **(patients 1-20)**

Twenty patients (17 M) aged 2 to 38 years, born to healthy unrelated parents, had a severe phenotype (Table 1). They include 3 sets of sibs. Age at diagnosis ranged between 12 months and 18 years. Two subjects had respiratory distress at birth (pts 11 and 18). All patients came to medical attention as infants because of psychomotor disability and hypotonia, roving eye movements and strabismus and dysmorphisms. In 55 % (11/20) early additional features in the

first years of life included failure to thrive and recurrent increases of serum transaminases. Abnormal fat pads and inverted nipples were reported in five patients (2,3,11,18,20). Severe developmental disability was observed in all patients: 90 % (18/20) acquired to walk only with support between 7 and 8 years. Acquisition of meaningful words occurred by the age of 3 years, 80 % (16/20) of the patients had severe intellectual disability.

At the time of the study hallmark dysmorphism included peculiar facial appearance with low sloping forehead, strabism, prominent jaw, and large ears and ear lobules (Figure 1). Also, skeletal deformities (short keel thorax and kyphoscoliosis) and some degree of abnormal subcutaneous fat distribution were observed. Thirteen patients had microcephaly, in four patients head circumference was in the normal range while information on this was not available in 3 other subjects. In all subjects neurological examination showed visual impairment, severe and generalized ataxia, inability to stand and to walk independently, distal muscle atrophy and absence of deep tendon reflexes. The most severely affected patients had tetraparesis with poor head control, motor stereotypies and not-purposeful limb movements (pts 18 and 19).

Electrophysiological studies in twelve patients showed reduced amplitude of electroretinogram (ERG) and visual evoked potentials (VEPs) (pts. 1-5, 16, 18-20) or absence of scotopic ERG (pts 7,11, 15), sensorineural abnormalities at brainstem auditory evoked potentials (BAEPs) (pts. 7, 11, 20) and mixed peripheral neuropathy (pts 1-4, 7, 11, 16, 18-20). Cranial Magnetic Resonance Imaging (MRI) showed severe atrophy of cerebellar hemispheres and vermis composed mostly of the superior portions and atrophy of the brainstem (especially the pons and inferior olives). The fourth ventricle was enlarged and appeared to communicate with a large posterior fossa cyst. (Figure 3 a-b). Follow-up neuroimaging (range 9 months-14 years) in seven studied subjects showed progression with volume loss of the cerebellum and pons.

Five patients (6, 11, 15, 17-18) had generalized and/or myoclonic fits. Epileptic seizures in these subjects started at variable age ranging from 4 months to 17 years. All received antiepileptic drug treatment, in three patients seizures were controlled with polytherapy, none was seizure free at the time of the study. In addition, four subjects had febrile seizures during infancy (pts 1-3, 12) and one had isolated paroxysmal electroencephalography (EEG) anomalies (pt 7). Among 17 patients in whom this information was obtained, nine had stroke-like events with age at onset ranging from 24 months to 18 years. These manifested as recurrent episodes of stupor and/or coma, transient

hemiparesis or hemiplegia, amaurosis, painful paraesthesias, headache and aphasia. Time of restitution *ad integrum* varied between 1 day and 2 years. Associated events were fever and infectious episodes, subtle head trauma, alcohol ingestion and invasive cerebral investigations (angiography).

All patients had abnormal serum coagulation factor and inhibitor levels (decreased Factor XI, AT-III, PC) and some degree of multisystem involvement with variable manifestations that included enteropathy (pts. 18, 20), hypogonadism in females, cryptorchism (pt. 11), nefrocalcinosis (pt. 19), renal cortical hyperechogenicity (pts. 15,19), recurrent skin infections (pt. 2), deep venous thrombosis (pts. 4,11,19) and severe scoliosis and osteoporosis that required treatments (pts. 1-2, 4-5, 11, 17-19) .

One patient (pt. 11) deceased at age 21 years during a febrile illness with acute respiratory failure. All other patients are alive, wheel-chair bound and supervised with impaired daily life autonomy.

Mild Phenotype **(patients 21-37)**

Seventeen patients (7 M) with age ranging from 3 to 67 years had a milder phenotype (Table 2). Age at diagnosis ranged from 9 months to 58 years. There were 5 sib pairs from four unrelated families. Consanguinity was ascertained in one family (parents of patients 21-22). Two sisters (pts 23-24) affected with intellectual disability and ataxia were diagnosed at 40 and 58 respectively when PMM2-CDG was established in their nephews (pts 21-22).

First clinical signs were developmental disability and cerebellar signs including ataxia, hypotonia and strabismus. Motor milestones were mildly delayed with 82 % (14/17) patients being able to walk without support between 19 and 40 months. First words production occurred at an age ranging from 10 and 18 months and first sentences before the age of 30 months. Eleven patients ranged between borderline cognitive abilities and mild intellectual disability. Minor facial dysmorphism including epichantus and a broad nasal bridge were occasionally reported. Only three patients had increased serum transaminase levels in the first year of life, one had failure to thrive and growth retardation. Severe enteropathy was diagnosed in one patient and controlled with a gluten-free diet.

At the time of the study, the hallmark clinical features were cerebellar ataxia, dysarthria and dysmetria associated with mild or moderate intellectual disability. Almost all patients (14 out of 17) are able to ambulate independently and display sufficient autonomy although with impaired motor coordination (Figure 2). None has epilepsy and/or stroke-like episodes. Six subjects have areflexia with reduced motor nerve conduction velocity and amplitude. Eight patients (21-26; 32-33) have some degree of visual defects with retinopathy and abnormal ERG/VEP.

Fourteen studied patients had cerebellar atrophy with mild to moderate atrophy of the cerebellar vermis and hemispheres. Only one of these patients had severe cerebellar atrophy with a large fluid space behind the cerebellum. T2/flair hyperintensity of the cerebellar cortex or dentate nuclei, and pontine atrophy were additional findings. In two subjects cerebellar atrophy was diagnosed prenatally. Six patients had follow-up neuroimaging studies with signs of progression during a period ranging from 12 months to 15 years (Figure 3 c-d).

Fourteen studied subjects had coagulopathy including antithrombin III, protein C and factor XI deficiencies. Severe scoliosis was found in four (pts. 3-4; 29-30), one had vertebral arthrodesis at the age of 13 years (pt. 29). Primary amenorrhea was diagnosed in females at due age. All patients are alive. One patient is 67 years. She is wheel-chair bound with behavioral disturbances and a distinct physical appearance including coarse face, trunk obesity and hirsutism, severe kyphoscoliosis, valgus knees and varus feet. Twelve young patients attend mainstream schools, use social networks and practice an integrated life style.

Genotype

Tables 1 and 2 show the results of the PMM2 mutation screening in our series. All subjects were compound heterozygotes for mutations in the PMM2 gene and 22/37 (seventeen unrelated families) had the 422G>A (R141H) missense mutation. The 95TA>GC (L32R) mutation was the second most frequent mutation encountered in Italian patients and it occurred in 12 patients (seven unrelated families), all with a mild phenotype (table 2). Among patients with the severe phenotype, the 385G>A (V129M) and 691G>A (V231M) mutations were observed in three and two unrelated families respectively. The 722G>C (C241S) mutation was found in five patients from three unrelated families with a mild phenotype.

Glycosylation Analyses

In order to detect possible differences in glycosylation profile between patients with different clinical severity, we characterized serum Tf glycoforms by MALDI-MS (Figure 4). Fifteen patients, (severe phenotype n: 8, aged 10-38; mild phenotype n: 7, aged 3-67) were studied.

Tf has two N-glycosylation sites mainly occupied by biantennary complex-type glycans, with di-glycosylated Tf (2-gly Tf) being the main fraction in control serum. A significant difference in the decrease of di-glycosylated Tf was observed between mild (2-gly Tf 72.2±9.65) and severe (2-gly Tf 52.20±8.52) patients ($p< 0.0001$). Conversely, underglycosylated species were more increased in patients with the severe phenotype (1-gly Tf 33.93±4.81) than in patients with the mild one (1-gly Tf 21.98±6.61) ($p< 0.002$).

Discussion

We conducted a nationwide survey to characterize frequency, clinical features and associated glycosylation and molecular data in Italian PMM2-CDG patients. We detected 37 PMM2-CDG patients born to 29 unrelated families. In 2000 we reported the Italian experience on CDG including 10 PMM2-CDG patients, all born between 1973 and 1998 [18]. The present series includes a total of 18 patients born in the same 25-year period, 2 patients born before 1973 and additional 17 patients born between 1999 and 2011. The increment of detected subjects might reflect an increased awareness for CDG in our country. PMM2-CDG is the first described and by far the most common CDG with more than 800 patients known worldwide [19]. The highest incidence reported is 1 per 41,000 newborns in Denmark where the disease is not infrequent [20]. PMM2-CDG has a wide clinical spectrum and genetic heterogeneity illustrated by more than 170 patients reported in the medical literature between 1990 and 2012 [21]. Based on age-related clinical features a four-stage description of the disease has been proposed whereas another clinical classification distinguished between patients with a solely neurological form and those with a more extensive systemic involvement and life-threatening course [2,7,10]. We outlined two sets of patients based on the degree and the extent of neurological disease, PMM2 genotype and glycosylation data. In particular, all patients with severe phenotype were unable to walk unaided, 84 % had severe intellectual disability and 81 % microcephaly. Conversely, 82 % of mildly affected patients had independent ambulation, 64 % had borderline to mild intellectual disability and 35 %

microcephaly. Epilepsy and stroke-like events did not occur among patients with mild phenotype. The rate and extent of systemic involvement were more pronounced in severely affected patients. However, all patients in both groups had decreased plasma levels of coagulation factors and inhibitors. All female subjects, including one elderly woman who is among the few eldest patients reported so far, have had primary amenorrhea at due age and hypergonadotropic hypogonadism. Cerebellar atrophy is indicated as a clue to the diagnosis of PMM2-CDG also in patients with milder phenotypes [11]. Despite their different disease severity, all our patients had (olivo)ponto-cerebellar atrophy that was the hallmark clinical feature for the diagnosis. In PMM2-CDG, cerebellar or olivopontocerebellar atrophy may be detected in neonatal age [22]. In milder patients, brain MR might be unremarkable in the first years [23]. In our series, only in one case (patient 31) cerebellum had a nearly normal appearance at the age of 9 months; on follow-up there was progressive hypotrophy. Cerebellum involvement in PMM2-CDG is due to atrophy because of increased interfoliar spaces on the vermis and/or hemispheres that develop during the course of the disease and are progressive [24-25]. Cerebellar atrophy was progressive in all our patients with neuroimaging follow-up, independently from their degree of clinical severity. Reduced bulge of the pontine protuberance was observed at variable ages and seemed to occur in the more severe cases. Insights into the pathophysiology of cerebellar atrophy in PMM2-CDG suggest that cerebellar granule cells have a less significant response than cortical neurons to ER-stress induced by downregulation of PMM2 protein levels *in vitro*. This might explain prominent cerebellar neurodegeneration in PMM2-CDG patients [26].

Epilepsy is not considered a prominent feature of PMM2-CDG [11] and it occurred in almost 15 % of patients in our series. Defective glycosylation might induce epilepsy by several mechanisms including improper receptor structure and functioning. Notably, N- glycosylation influences biogenesis and channel gating of GABA A receptor that is the principal mediator of rapid inhibitory synaptic transmission in the human brain [27].

In PMM2-CDG more than one hundred gene mutations have been reported. As in other European countries, the most common mutation in Italian patients is the heterozygous R141H mutation representing 30 % of disease alleles. This is lower than reported elsewhere in Europe (43-50%) [28-29] , except for Spain (25%) [30]. The second most common mutation in the Italian population is

the L32R mutation (16 % of disease alleles). Besides these two, 14 other mutations were found, scattered over the gene.

It is noteworthy that heterozygous L32R occurs in 70 % (12/17) of patients with mild phenotype. It was found in association with different disease alleles R141H (3 families), V129M, T237M and F157S (one family each) thus supporting a significant association of L32R with the milder phenotype. Likewise, a few patients bearing L32R reported so far have less severe neurological manifestations than typically seen in PMM2-CDG [31-32]. L32R belongs to the *PMM2* misfolding mutations linked to structural instability of the PMM2 protein and susceptible of therapy by pharmacological chaperones [33]. Expression studies of the L32R mutant protein showed a 40 %-45 % activity compared to controls [34]. L32R was associated *in vivo* with a high residual PMM2 activity of almost 30 % in fibroblasts [31]. However, two of the present patients (21-22) and one of Coman's patients [32] bearing heterozygous L32R mutation and exhibiting the mildest neurological phenotypes, have a very low residual leukocyte PMM2 activity. It is plausible that issues such as the genetic background and environmental factors might influence the PMM enzyme activity. In this regard, we investigated the glycosylation profile of native serum Tf in our two patient samples with different disease severity by Maldi-MS. We found that the glycoproteomic differences parallel the clinical differences as the rate of underglycosylated Tf fractions is more pronounced in severely affected PMM2-CDG patients. Previous studies showed inter- and intrafamilial differences in glycosylation among PMM2-CDG patients with different disease severity, including a sib pair [35-36]. An association was found between the absence of occupation of serum Tf glycosylation sites analysed by MRM-LC-MS and disease severity supporting a correlation between the degree of glycosylation deficiency and clinical expression in PMM2-CDG [37]. A clear genotype-phenotype correlation was previously demonstrated in patients from Denmark with the R141H/F119L genotype considered to represent the severe end of the clinical spectrum of PMM2-CDG [20]. They had a uniform presentation with severe developmental delay, feeding problems, failure to thrive and multisystem involvement requiring hospitalization in the first years of life.

We conclude that the L32R mutation is particularly frequent in Italy and that it is associated with a rather mild neurological phenotype marked by preserved ambulatory ability and autonomy in a large set of PMM2-CDG patients. Based on present clinical characteristics and glycosylation findings patients with milder neurological phenotype bearing L32R misfolding mutation could

represent, among others, valuable candidates for development of pharmacological chaperones in PMM2-CDG. This study underlines the importance of considering PMM2-CDG in patients with even mild-borderline developmental disability and/or cerebellar atrophy of unexplained origin at all ages.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Legend to figures

1. PMM2-CDG severe phenotype. A) Patient 10 (age 8 years) B) Patient 5 (age 7 years). Craniofacial dysmorphism with microcephaly, prominent jaw and large ears.

2. PMM2-CDG mild phenotype. A) Patient 28 (age 8 years) B) Patient 35 (age 24 years). Note unremarkable physical appearance and preserved gross motor functions.

3. Different degree of cerebellar atrophy associated with *PMM2* mutation. Patients 3 (A) and 1 (B): R141H/N216I genotype (severe phenotype). Severe (olivo)-pontocerebellar atrophy with enlarged posterior fossa cyst. Patient 31: L32R / R123Q (mild phenotype). (C) Age 9 months: mild cerebellar volume loss with nearly normal appearance of the cerebellar vermis (D) Age 3 years: progressive cerebellar hypotrophy.

4. MALDI-MS analyses of serum Tf in a healthy control subject and in PMM2-CDG patients with different disease severity and genotype.
   With respect to control subjects, MALDI spectra from the PMM2-CDG patients show a decrease of the normal glycoform corresponding to diglycosylated Tf at 79.5 kDa. In addition, two underglycosylated glycoforms at 77.4 kDa and at 75.2 kDa are evident. Abnormal glycoforms correspond to mono-glycosylated Tf and a-glycosylated Tf, each lacking the entire N-glycan structure (2.2 kDa) at one or both N-glycosylation sites respectively. Serum Tf underglycosylated glycoforms are more increased in patients with severe phenotype.

Figure 1.

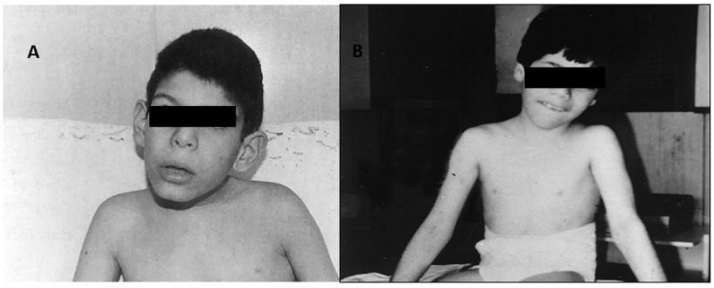

Figure 2.

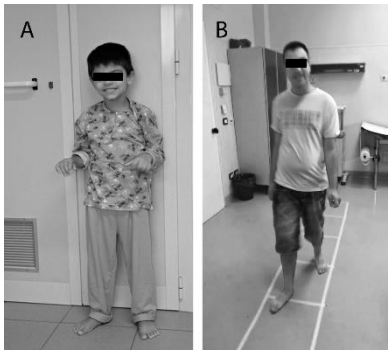

Figure 3.

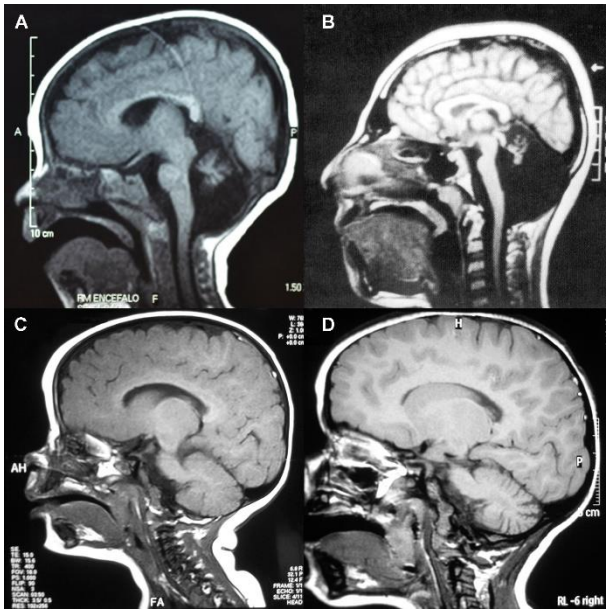

Figure 4.

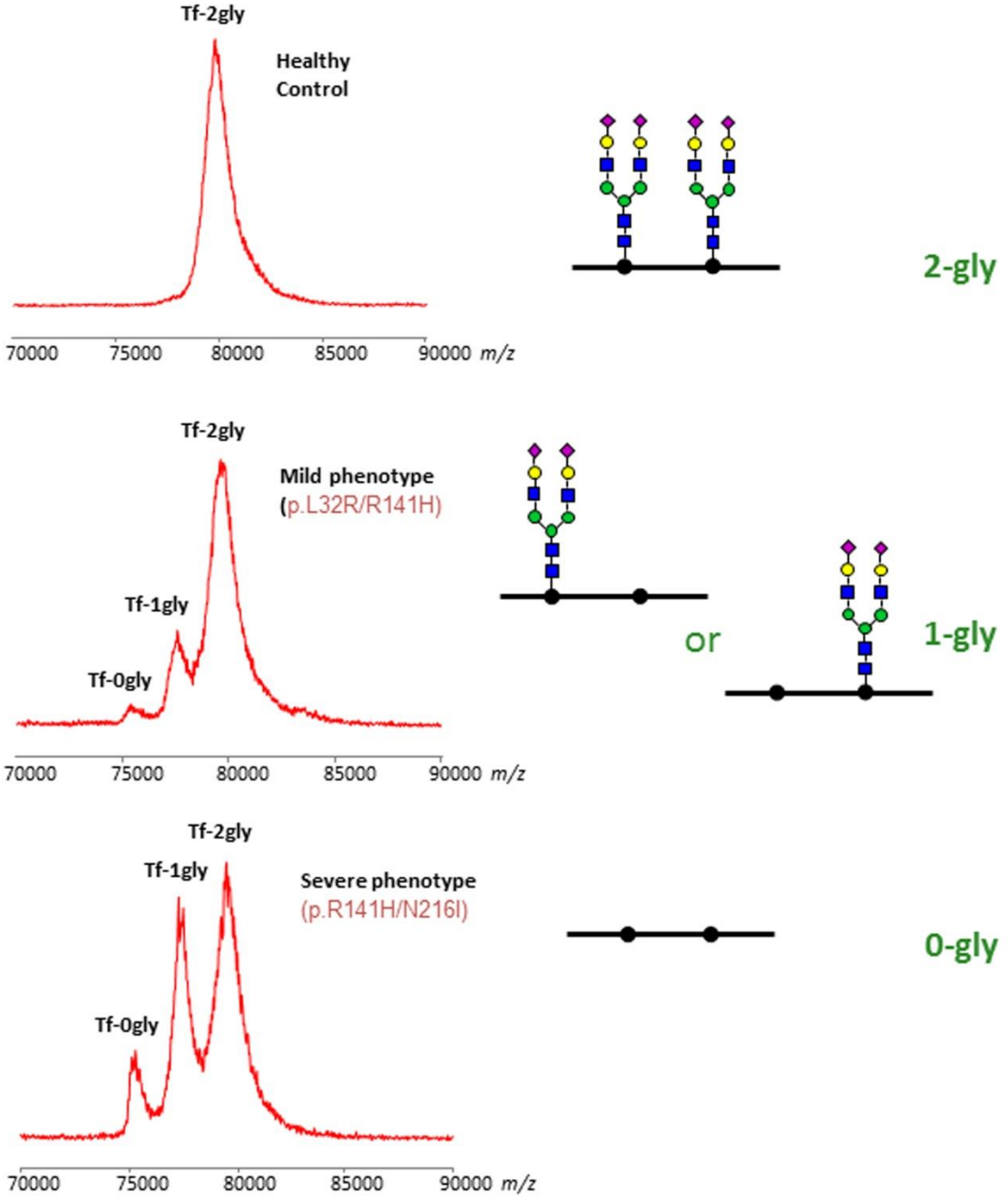
Tf-2gly
Healthy
Control
70000
75000
80000
85000
90000 m/z
2-gly
Tf-2gly
Mild phenotype
(p.L32R/R141H)
Tf-1gly
Tf-0gly
70000
75000
80000
85000
90000 m/z
or
1-gly
Tf-2gly
Tf-1gly
Severe phenotype
(p.R141H/N216I)
Tf-0gly
70000
75000
80000
85000
90000 m/z
0-gly

Table 1 Clinical features, PMM enzyme activity and genotype of PMM2-CDG patients (severe phenotype)

Patient	Sex/Age	Dysmorphic features	Intellectual disability	Unaided walk	Epilepsy	Stroke-like episodes	Visual defects	Microcephaly	Cerebellar hypoplasia	Extra-neurological features	°PMM activity	Genotype
1	M/38	+	Mo	−	−	+	+	+	Se	+	0.33(L)	R141H/N216I
2	F/35	+	Se	−	−	+	+	+	Se	+	0.25(L)	R141H/N216I
3	M/10	+	Se	−	−	+	+	+	Se	+	0.42(F)	R141H/N216I
4	M/36	+	Se	−	−	+	+	+	Se	+	2.61(F)	V129M/R141H
5	M/29	+	Se	−	−	+	+	+	Se	+	1.95(F)	V129M/R141H
6	M/11	+	Mo	−	+	−	+	−	Se	+	n.a.	V129M/R141H
7	M/24	+	Se	−	−	−	+	+	Se	+	0.33(F)	V231M/R141H
8	M/19	+	Se	−	n.a.	n.a.	+	+	Se	n.a.	0.89(F)	V231M/R141H
9	M/30	+	Se	−	−	−	+	+	Se	+	0.41(F)	I132T/R141H
10	M/23	+	Se	−	−	+	+	+	Se	+	n.a.	V231M/R141H
11	M/21	+	Se	−	+	+	+	+	Se	+	0.89(F)	G15E/D223N
12	M/12	+	Se	−	−	+	+	+	Se	+	n.a.	G15E/D223N
13	F/10	+	Se	−	n.a.	n.a.	n.a.	n.a.	Se	n.a.	n.a.	D223N/R141H
14	M/11	+	Se	−	n.a.	n.a.	+	n.a.	Se	+	n.a.	V231M/F157SE
15	M/8	+	Mo	−	+	−	−	n.a.	Se	−	n.a.	L193V/T237R
16	M/18	+	Mi	−	−	−	+	−	Se	+	1.65(F)	T237M/R141H
17	M/8	+	Mo	−	+	−	−	+	Se	+	n.a.	L193V/T237R
18	M/7	+	Se	−	+	−	+	−	Se	+	0.27(F)	V129M/R141H
19	M/38	+	Se	−	−	−	+	−	Se	+	0.74(F)	I132T/I132T
20	M/2	+	Se	−	−	−	+	+	Se	+	n.a.	V129M/R141H

Sib pairs: pts 1-2; 4-5; 11-12; M: male; F: female; Se: severe; Mo: moderate; Mi: mild; +: present; -: absent; n.a.: not assessed; °PMM activity (mU/mg protein); (L): leukocytes (normal range: 0.71-2.16); (F): fibroblasts (normal range: 3.6-5.4)

Table 2 Clinical features, PMM enzyme activity and genotype of PMM2-CDG patients (mild phenotype)

Patient	Sex/Age	Dysmorphic features	Intellectual disability	Unaided walk	Epilepsy	Stroke-like episodes	Visual defects	Microcephaly	Cerebellar hypoplasia	Extra-neurological features	°PMM activity	Genotype
21	F/13	−	Mi	+	−	−	+	+	Mo	+	<0.1(L)	L32R/R141H
22	F/3	−	Mi	+	−	−	+	+	n.a.	+	<0.1(L)	L32R / R141H
23	F/67	+	Mo	−	−	n.a.	+	+	n.a.	+	0.23(L)	L32R / T237M
24	F/49	+	Mo	+	−	n.a.	+	+	n.a.	+	0.42(L)	L32R / T237M
25	F/19	−	Mi	+	−	−	+	−	Mo	−	n.a.	L32R / F157S
26	M/22	−	Mi	−	−	−	+	−	Se	−	n.a.	L32R / F157S
27	M/12	−	Mi	+	−	−	−	−	Mo	+	n.a.	T237R / C241S
28	M/14	−	Mi	+	−	−	−	−	Mi	+	n.a.	L32R / R141H
29	F/17	−	Mi	+	−	−	−	−	Mo	+	1.7(F)	C241S / R141H
30	M/15	−	Mi	+	−	−	−	−	Mo	−	1.8(F)	C241S / R141H
31	F/11	−	Mi	+	−	−	−	+	Mi	+	2.2(F)	L32R / R123Q
32	M/36	−	Mo	+	−	−	+	−	Mo	−	0.2(L)	L32R / V129M
33	F/30	−	Mo	+	−	−	+	−	Mo	−	0.3(L)	L32R / V129M
34	M/10	−	Mo	−	−	−	−	+	Mo	−	3.35(F)	L32R / R141H
35	M/24	+	Mo	+	−	−	−	−	Mo	−	n.a.	L32R / R141H
36	F/3	−	−	+	−	−	−	−	Mo	−	n.a.	*A108V/* (c.511dupA)
37	F/6	+	−	+	−	−	−	−	Mo	−	n.a.	C241S / R141H

Sib pairs: pts 21-22; 23-24; 25-26; 29-30; 32-33. M: male; F: female; Se: severe; Mo: moderate; Mi: mild; +: present; -: absent; n.a.: not assessed;

°PMM activity (mU/mg protein); (L): leukocytes (normal range: 0.71-2.16); (F): fibroblasts (normal range: 3.6-5.4)