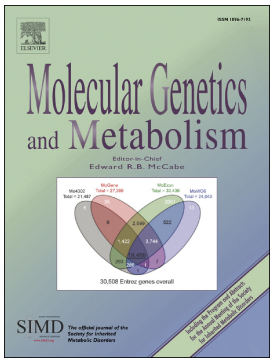

A founder variant in Tunisian PMM2-CDG patients: An integrated clinical, radiological, biochemical, and genetic study

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A Founder Variant in Tunisian PMM2-CDG Patients: An Integrated Clinical, Radiological, Biochemical, and Genetic Study.

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Abstract

PMM2-CDG is the most common congenital disorder of glycosylation, characterized by a broad phenotypic spectrum involving the nervous system and multiple other organ systems. The disorder is caused by biallelic variants in the *PMM2* gene, leading to impaired glycosylation of proteins.

Our objective was to provide a detailed clinical characterization and define the mutational spectrum of PMM2-CDG in the Tunisian population. We conducted a retrospective study on patients with genetically confirmed PMM2-CDG, followed between 2005 and 2024. Ten patients from six unrelated Tunisian families were enrolled. All presented with neurological symptoms, including psychomotor delay (10/10), cerebellar ataxia (9/10) and strabismus (9/10). Brain MRI revealed cerebellar atrophy in all patients. Dysmorphic features were common including almond-shaped eyes (9/10), large mouth (6/10), and thin upper lip (6/10). Skeletal anomalies were observed in 9/10 patients. Peripheral neuropathy was confirmed in 6/7 patients. Laboratory analyses revealed elevated transaminases (6/10), hypocholesterolemia (7/10), elevated LDH (7/10), hypoalbuminemia (2/6), and IgA deficiency (3/5). Renal anomalies included hyperechogenicity (2/9) and a duplicated collecting system (1/9). Genetic analysis revealed a homozygous variant NM_000303.3(*PMM2*): c.395T>C; p.(Ile132Thr) in all patients. Haplotype analysis of the *PMM2* locus showed that all 6 families shared an identical allele. In conclusion, this is the first study to characterize the clinical and genetic profile of PMM2-CDG in the Tunisian population. Despite a shared genotype, patients exhibited moderate neurological phenotypes with inter- and intrafamilial variability. The recurrent homozygous c.395T>C; p.(Ile132Thr) variant and identical haplotype confirm a founder effect in the Tunisian population.

Keywords

PMM2-CDG, Congenital disorder of glycosylation, genotype-phenotype correlation, *PMM2* gene, founder effect.

Author Contributions

The study concept and design were developed by L.K., T.B., and I.K. Clinical investigations of the patients were performed by I.K., L.K., T.B., A.Z., H.K., Z.M., and H.B. Radiological interpretation was provided by S.N. Transferrin isoforms analysis was conducted by M.E. Western blot analysis of serum glycoproteins, PMM leukocyte activity, and molecular investigations were performed by S.A., A.B., E.L., T.D., and S.V.B. The original draft was written by L.K. and T.B., and critically reviewed, edited, and validated by I.K., A.B. and S.V.B. All authors read and approved the final version of the manuscript and agreed to its submission.

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Conflict of Interest

No conflicts.

Patient consent statement:

Authors have obtained written informed consent from patients.

Data Availability Statement

The data supporting the findings of this study are available within the article.

1. Introduction

Congenital disorders of glycosylation (CDG) are a clinically and genetically heterogeneous group of disorders resulting from abnormal glycosylation of glycoconjugates. PMM2-CDG, the most frequent type of CDG, is caused by pathogenic variants in the *PMM2* gene, leading to reduced activity of phosphomannomutase 2 (PMM2) [1]. PMM2-CDG is a multisystem disorder with a highly variable phenotype. Neurological symptoms are frequent including cerebellar ataxia, strabismus, intellectual disability, and peripheral neuropathy [2,3]. PMM2-CDG is confirmed by measurement of PMM2 activity in fibroblasts or leukocytes and/or by identifying pathogenic variants in the *PMM2* gene [2].
The aim of this study was to provide a detailed clinical characterization and define the mutational spectrum of PMM2-CDG in the Tunisian population.

2. Methods

We conducted a retrospective cohort study of patients with genetically confirmed PMM2-CDG. All patients were followed in the Department of Pediatric Neurology at the National Institute Mongi Ben Hmida of Neurology in Tunis (Tunisia), between 2005 and 2024.

2.1. Clinical Evaluation

All patients were examined by both a pediatric neurologist and a clinical geneticist. Detailed genealogical data were collected for all families. Raven's Progressive Matrices (PM47) was used to assess the intelligence quotient (IQ) in children older than 5 years. In addition, an ophthalmologic evaluation was performed in some patients.

2.2. Neurological Assessment

Neurological work-up included brain magnetic resonance imaging (MRI) and electroneuromyography (ENMG). MRI was performed in all patients, with four undergoing follow-up imaging. The majority of patients underwent ENMG.

2.3. Laboratory and radiological investigations

Multiple laboratory investigations were performed, including biochemical tests (renal and liver function tests, lipid profile, serum protein levels, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), serum glucose, hematological tests (complete blood count, prothrombin time, and measurement of anticoagulant proteins such as antithrombin III, protein C, and protein S), and immunological tests (IgA quantification), which were carried out either in all patients or in a subset. Serum transferrin isoelectric focusing was performed in half of the patients. Western blot analysis of serum glycoproteins (transferrin, alpha-1-antitrypsin, haptoglobin, and alpha-1-acid glycoprotein) was performed in all families. PMM leukocyte activity was assessed in more than half of the

patients. Radiological examinations, including abdominal and renal ultrasound and echocardiography, were performed in some patients.

2.4. Genetic Analyses

Blood samples were collected for DNA extraction. After PCR amplification and purification, bidirectional sequencing of the exons and flanking intron-exon junctions of the *PMM2* gene was performed in all patients. When available, parental DNA samples were also tested for the identified variant. Haplotype analysis was performed on each index case of the 6 families. All were typed for 7 extragenic microsatellite markers 12538882, 12466423, 12457635, D16S513, D16S768, D16S3020 and D16S406. Primer's sequence was obtained from Genome Data Base. The PCR products were analyzed on an ABI PRISM 3500 XL genetic analyzer (Applied Biosystems™).

2.5. Ethical Considerations

This study was conducted in accordance with the ethical principles for medical research outlined in the Declaration of Helsinki. Data were anonymized to ensure confidentiality, and written informed consent was obtained from all families prior to genetic testing. This study was approved by the Ethics Committee of the National Institute Mongi Ben Hmida of Neurology (Ethics approval No. 40/25).

3. Results

Ten patients (P1-P10) from six unrelated families (F1-F6) (**Figure 1**) were included in this study. At the time of the first consultation, their ages ranged from 1 to 13 years (mean age: 6.1 years). The cohort included three sporadic cases, a pair of monozygotic twins, three siblings, and two sisters. At the time of the last follow-up, the mean age was 10.4 years (range: 2-24 years). Parental consanguinity was observed in five of the six families. Normal pregnancies and deliveries were reported in seven patients. One patient (P6) was delivered by cesarean section due to acute fetal distress. Three patients (P1, P6, and P7) presented respiratory distress at birth. Two patients (P2 and P6) had low birth weight. First signs of PMM2-CDG were identified before the end of the first year in three patients. Psychomotor delay was the principal presenting feature in all patients, with hypotonia additionally observed in four of them. All patients experienced a language and walking delay. Three patients acquired the ability to walk with assistance at a mean age of 5.5 years. Cerebellar ataxia and strabismus were present in nine patients. Gait ataxia appeared to remain stable over time. Nystagmus was present in four patients, while fundus examinations, performed in five patients at a mean age of 6.4 years, were all normal. Intellectual disability was present in the five tested cases with mild to moderate severity. Trunk stereotypies and a jovial mood were each noted in four patients. Signs of peripheral neuropathy were reported in six patients, mainly involving abolished osteotendinous reflexes and distal amyotrophy. Acquired microcephaly was found in eight patients, with head circumferences ranging from -2 to -3 standard deviations (SD). Two patients had delayed puberty with

absent secondary sexual characteristics (**Figure 2**). Dysmorphic features were observed in several patients, including almond-shaped eyes (9/10), a wide mouth (6/10), and a thin upper lip (6/10). Orange peel skin was noted in three patients (mean age: 11.6 years), and inverted nipples were observed in two patients at ages 12 months and 6.5 years. Abnormal fat distribution was observed in three patients (**Figure 2**). Skeletal abnormalities were present in nine patients. P5 had kyphosis, and P4 and P7 developed scoliosis over time (**Figure 2**). Brain MRI, performed at a mean age of 4.6 years, revealed cerebellar atrophy in all patients (**Figure 3**). Follow-up brain MRI was conducted in four patients after a three-year interval, showing no progression of the cerebellar atrophy. Nerve conduction velocity (NCV) was assessed in seven patients, with ENMG performed between 22 months and 16 years of age. One had normal NCV, while six exhibited features of demyelinating neuropathy. Among the six patients with demyelinating neuropathy, three (P4, P5, and P7) initially showed normal NCV results at 13, 9, and 13 years of age, respectively. Subsequent evaluations revealed progressive demyelination at 16, 11, and 15 years, respectively. Abdominal ultrasound was performed in 9 patients and showed hepatomegaly in P3 and renal abnormalities in P2 and P3. Cardiac echocardiography was performed for only three patients and showed no abnormalities. Laboratory abnormalities included elevated transaminases (6/10), hypocholesterolemia (7/10), hypoalbuminemia (2/6), and elevated LDH levels (7/10). Low IgA levels were detected in three out of five patients tested. Anemia was identified in 4/10 patients. Prothrombin time, measured in six patients, was at the upper limit of normal in one (Quick's time: 17.6 s vs. control: 14.2 s; prothrombin ratio: 71%; international normalized ratio (INR): 1.24). Antithrombin III deficiency was identified in both tested patients; one also had a protein C deficiency, and the other had a protein S deficiency. Isoelectric focusing of serum transferrin, performed in patients P1-P5, revealed an abnormal pattern, with increased asialotransferrin and disialotransferrin. Western blot analysis of serum glycoproteins (transferrin, alpha-1-antitrypsin, haptoglobin, and alpha-1-acid glycoprotein), performed in patients P1-P9, revealed bands of lower molecular weight compared to healthy controls, consistent with a diagnosis of type I CDG (**Figure 4**). PMM leukocyte activity, assessed in six patients (P1-P6), demonstrated a marked deficiency, with values ranging from 0.6 to 0.9 U/g (Normal > 6 U/g). PMM activity in the tested parents showed partial deficiency, with values between 2.4 and 4.8 U/g. *PMM2* gene sequencing confirmed PMM2-CDG diagnosis by revealing a homozygous variant in exon 5: NM_000303.3(*PMM2*): c.395T>C; p.(Ile132Thr) in all patients. This variant was identified in the heterozygous state in the tested parents. Haplotype analysis of the *PMM2* locus indicated that all 6 families shared an identical allele for the 7 extragenic microsatellite markers studied. The median age at molecular diagnosis was 6.7 years. These results are summarized in **Table 1.**

4. Discussion

This study provides a comprehensive overview of the clinical, biological, and radiological features observed in ten Tunisian patients with genetically confirmed PMM2-CDG. The phenotypic

presentation in PMM2-CDG affected patients ranges from an isolated neurological involvement to a multisystemic form combining both neurological and visceral manifestations [3]. Our patients exhibited the characteristic signs of the neurological form of PMM2-CDG. Neurological manifestations were dominated by hypotonia, typically present from birth, and by psychomotor delay which was the main reason for initial consultation in all cases.

Cerebellar ataxia has been reported in 95 to 100 % [3], and was observed in nine out of ten patients in our cohort. In all cases, it appeared clinically non-progressive, supporting the hypothesis of a developmental rather than a degenerative process [4]. This ataxia is attributed to defective N-glycosylation, which impairs neuronal migration, synaptogenesis, and axonal guidance during brain development. These mechanisms particularly affect the cerebellum, leading to congenital hypoplasia and progressive dysfunction. This clinical observation aligns with the typical PMM2-CDG neuroimaging pattern, marked by vermian-dominant cerebellar atrophy and pontine involvement [3]. These abnormalities can be detected as early as in infancy, reinforcing the idea of prenatal onset of the disease [5]. A correlation between the severity of cerebellar symptoms and imaging findings has been suggested [6]. In some cases, the cerebellar white matter appears slightly hyperintense compared to the supratentorial white matter, producing the characteristic “bright cerebellum” sign [5]. In our cohort, cerebellar atrophy was present in all patients. This radiological sign, along with psychomotor delay and strabismus, led us to suspect PMM2-CDG diagnosis. In addition, one patient presented with a Dandy-Walker variant, a rare association previously reported in only one case [7] while another patient showed pontine atrophy.

Additional neurological features include a progressively variable peripheral neuropathy, often observed during the second year of life in patients with PMM2-CDG. This neuropathy typically affects motor nerves, with either axonal or demyelinating patterns [4]. Its severity tends to increase with age [8]. In our cohort, ENMG performed on siblings P4, P5 and P7 at 13, 9, and 13 years of age, respectively, was initially normal, but subsequently revealed demyelinating neuropathy at 16, 11 and 15 years, respectively. However, P6, the younger brother of P4 and P5, showed signs of neuropathy as early as age 4, highlighting the intrafamilial variability in onset and progression of peripheral nerve involvement. Intellectual disability is a major neurological feature of PMM2-CDG, with variable severity [9]. IQ typically ranges from 40 to 70. Individuals with borderline intellectual function and normal development have been described [4,10,11]. The children usually are extroverted and cheerful [9]. Seizures and stroke-like episodes may occur [2]. In our cohort, all tested patients presented with mild to moderate intellectual disability, and a jovial mood was noted in four out of ten patients. No patient experienced seizures or stroke-like episodes.

Ophthalmological manifestations of PMM2-CDG include strabismus, saccadic eye movements, nystagmus, retinitis pigmentosa and refractive errors [8,12]. Strabismus is among the most frequent initial signs of the disease and was observed in nine patients in our cohort, with associated nystagmus

in four cases. Retinitis pigmentosa occurs more frequently in adult patients and is more reliably detected by electroretinography than by fundoscopic examination [4]. This may explain its absence in our cohort, as fundus examination was performed in only four pediatric patients, and no long-term ophthalmological follow-up was conducted.

Concerning growth, postnatal growth decline is commonly observed in children with PMM2-CDG [2]. In our cohort, stagnation of head circumference growth, leading to microcephaly, was noted in eight patients. The exact mechanism underlying growth failure in PMM2-CDG remains unclear, though it has been suggested to involve disruption of the growth hormone-insulin-like growth factor 1 (GH-IGF-1) axis [13]. However, no hormonal assessment was available in our cohort to support this hypothesis. Dysmorphic features have been frequently reported in patients with PMM2-CDG. They mainly include a prominent forehead, large ears with prominent lobules, almond-shaped eyes, a thin upper lip, a prominent jaw, and long, slender fingers and toes [2]. In our cohort, the most observed features were almond-shaped eyes (9/10 patients), a large mouth (6/10), and a thin upper lip (6/10). In addition to facial dysmorphism, inverted nipples have been reported in up to 50% of patients with PMM2-CDG [1, 2]. Abnormal subcutaneous fat distribution, particularly over the buttocks and suprapubic region, has also been described in 25% to 91% of cases [2]. Both features typically disappear with age. In our cohort, inverted nipples were observed in two patients, at 12 months and 6.5 years of age, respectively. Abnormal fat distribution with orange peel skin was also noted in three other patients aged 8 to 13 years.

Beyond neurological impairment, systemic involvement is common in PMM2-CDG, affecting endocrine, hematological, immunological, skeletal, and renal systems [1,3]. Concerning the endocrine system, hypogonadism is a characteristic feature of PMM2-CDG, often presenting as premature ovarian failure in females [4]. Pubertal abnormalities in males are less frequently reported, although small testicular volumes have been described [2]. In our cohort, two female patients presented absent pubertal development. Abnormal thyroid function is observed in approximately 75% of patients with CDG, likely due to defective glycosylation of thyroid-stimulating hormone (TSH) and thyroid-binding globulin [4]. However, thyroid function was normal in all tested patients in our cohort. Other laboratory abnormalities reported in PMM2-CDG include elevated transaminases, hypocholesterolemia, hypoalbuminemia, hypoglycemia, coagulation factor deficiencies (factors IX and XI), deficiencies in natural anticoagulants (antithrombin III, protein C, and protein S), and altered immunoglobulin levels [2]. In our cohort, elevated transaminases were observed in six out of ten patients, and hypocholesterolemia was found in seven out of ten. Hypoalbuminemia was noted in two out of six tested patients. Anemia was identified in four out of ten patients. A slightly prolonged prothrombin time, at the upper limit of normal, was observed in one of the six patients tested. Antithrombin III deficiency was detected in two tested patients; among them, one had a protein C deficiency and the other a protein S deficiency. Low IgA levels were found in three out of five tested

patients. Additionally, elevated LDH levels were observed in seven out of ten patients. To our knowledge, this abnormality has been previously reported in only one PMM2-CDG patient [14].

Musculoskeletal abnormalities, such as osteopenia, thoracic deformities, kyphosis, scoliosis, and joint malformations, are frequently reported [1]. In our study, four patients had pectus excavatum, one patient had kyphosis and two developed scoliosis during the disease course.

Renal involvement in PMM2-CDG has been more commonly reported in the severe infantile form associated with multivisceral manifestations [9]. Reported abnormalities on renal ultrasound include multicystic kidneys, nephromegaly, increased echogenicity, and altered corticomedullary differentiation [15]. In our cohort, renal hyperechogenicity was observed in the twin sisters P2 and P3. Additionally, renal ultrasound in patient P2 revealed a duplicated left collecting system. To our knowledge, this anomaly has not been previously reported in association with PMM2-CDG and may therefore represent a coincidental finding. Indeed, duplication of the urinary collecting system is the most common malformation of the upper urinary tract, with a prevalence of 0.7-4% and a female predominance [16]. An autosomal dominant mode of inheritance with incomplete penetrance has been suggested, which could explain the absence of this anomaly in the monozygotic twin sister [17].

Other findings such as congenital cardiac anomalies and hypertrophic cardiomyopathy with transient myocardial ischemia have been reported in patients with PMM2-CDG [2]. Only three patients underwent echocardiogram, which did not reveal any abnormalities.

PMM2-CDG is an autosomal recessive disorder caused by pathogenic variants in the *PMM2* gene, located on chromosome 16p13.2. This gene encodes phosphomannomutase 2, a cytosolic enzyme that catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate, a crucial step in N-linked glycosylation [1].

PMM2-CDG is typically suspected based on a type I transferring glycoforms pattern detected in serum and confirmed by the identification of biallelic pathogenic variants in *PMM2* through molecular genetic testing. Enzymatic activity measurement can be used as part of the diagnostic confirmation. However, it may be normal in some cases [18].

In our cohort, isoelectric focusing of serum transferrin (performed in patients P1-P5) and Western blot analysis of serum glycoproteins (performed in patients P1-P9) revealed a type I CDG pattern (**Figure 4**). PMM leukocyte activity, assessed in six patients, demonstrated a marked deficiency, with values ranging from 0.6 to 0.9 U/g (Normal > 6 U/g). *PMM2* sequencing confirmed the diagnosis in all patients.

Pathogenic variants in *PMM2* lead to defective glycosylation affecting multiple systems. To date, over 100 pathogenic variants have been identified. Most patients are compound heterozygotes, typically carrying one inactivating and one hypomorphic variant [19]. The most common variant is c.422G>A;

p.(Arg141His), present in approximately 45-60% of alleles in affected individuals. This variant is notably never found in homozygosity, likely due to lethality [2].

Several founder variants have been described in specific populations, such as c.422G>A/c.357C>A; p.(Arg141His)/p.(Phe119Leu) in Scandinavia, c.193G>T; p.(Asp65Tyr) in the Iberian Peninsula, c.415G>A; p.(Glu139Lys) in French families, and c.95TA>GC; p.(Leu32Arg) in Italian patients [8, 20-22].

In our cohort, all tested patients carried the homozygous c.395T>C; p.(Ile132Thr) variant in exon 5, and the tested parents of patients were confirmed to be heterozygous carriers. Although this variant has previously been reported in other populations, it was mostly observed in compound heterozygosity with variants such as c.422G>A; p.(Arg141His), c.368G>A; p.(Arg123Gln), or c.58C>T; p.(Pro20Ser)/c.66+1G>T [8, 23]. Only two cases with a homozygous c.395T>C variant have been described so far: one Italian patient with a severe phenotype [20], and, more recently, a Tunisian family with a mild neurological presentation, diagnosed in adulthood by clinical exome sequencing [24]. The identification of the same homozygous variant in all Tunisian patients strongly suggests a founder effect, which has been confirmed by our haplotype analysis (**Table 2**). The high rate of consanguinity observed among the studied families likely contributes to the homozygous occurrence of the *PMM2* c.395T>C; p.(Ile132Thr) variant and may increase the overall burden of recessive alleles. This population structure could also favor the presence of additional rare variants in other genes involved in protein glycosylation or related pathways, potentially modulating the clinical phenotype. Future studies using targeted panels or exome/genome sequencing may help identify modifier variants and refine genotype-phenotype correlations.

Establishing a clear genotype-phenotype correlation in PMM2-CDG remains challenging due to significant clinical variability and the broad spectrum of variants. Nevertheless, some correlations have been documented. Severe phenotypes are frequently associated with genotypes such as c.422G>A/c.357C>A; p.(Arg141His)/p.(Phe119Leu), c.563A>G/c.422G>A; p.(Asp188Gly)/p.(Arg141His), and c.691G>A; p.(Val231Met). In contrast, milder neurological phenotypes have been linked to combinations such as c.422G>A/c.395T>C; p.(Arg141His)/p.(Ile132Thr), c.422G>A/c.415G>A; p.(Arg141His)/p.(Glu139Lys), and C-terminal variants like c.653A>T; p.(His218Leu), c.710C>T; p.(Thr237Met), and c.722G>C; p.(Cys241Ser). These variants are believed to retain partial enzymatic activity, which may explain the attenuated clinical presentations observed in some cases [23,25]. Recent comprehensive genotype-phenotype studies in PMM2-CDG provided insightful correlations between mutation types, enzyme function, and clinical outcomes. In a cohort of 137 patients, variants were grouped according to their mechanistic impact on catalysis, folding, or dimerization. Variants affecting folding or dimerization, such as

p.Val231Met, were associated with more severe disease, whereas variants like p.Cys241Ser were correlated with milder phenotypes [18].

In our cohort, all patients exhibited a moderate neurological phenotype of PMM2-CDG, potentially associated with the c.395T>C; p.(Ile132Thr) variant with notable inter- and intrafamilial variability. The Ile132Thr variant in PMM2-CDG involves the substitution of a hydrophobic isoleucine with a polar threonine in the cap domain of the protein. This substitution mildly destabilizes local folding and perturbs the dynamic motions needed for proper cap closure, but it preserves the overall structure and residual enzymatic activity. This mild destabilization results in a variant with relatively mild functional deficits and less severe clinical symptoms compared to other pathogenic variants [26].

Life expectancy in PMM2-CDG is highly variable and depends on the severity of multisystem involvement. While severely affected infants may die in early childhood, many patients with milder variants such as I132T survive into adolescence or adulthood. In our cohort, all individuals were alive at last follow-up, but the short observation period does not allow definitive conclusions regarding long-term survival [8].

5. Conclusion

This study provides the first comprehensive clinical, biochemical, and molecular characterization of PMM2-CDG in a Tunisian cohort. All patients harbored the homozygous c.395T>C; p.(Ile132Thr) variant and similar haplotype, confirming a founder effect in this population. Despite a shared genotype, patients demonstrated a moderate neurological phenotype with inter- and intrafamilial variability, highlighting the complex correlation between genotype and phenotype in PMM2-CDG. Targeted testing of the c.395T>C variant may serve as a rapid preliminary step within a broader molecular diagnostic strategy integrating comprehensive gene analysis in Tunisian patients. Timely diagnosis and early molecular confirmation of PMM2-CDG are essential for early management, accurate genetic counseling, identification of eligible patients for clinical trials, and future access to emerging targeted or repurposed therapies.

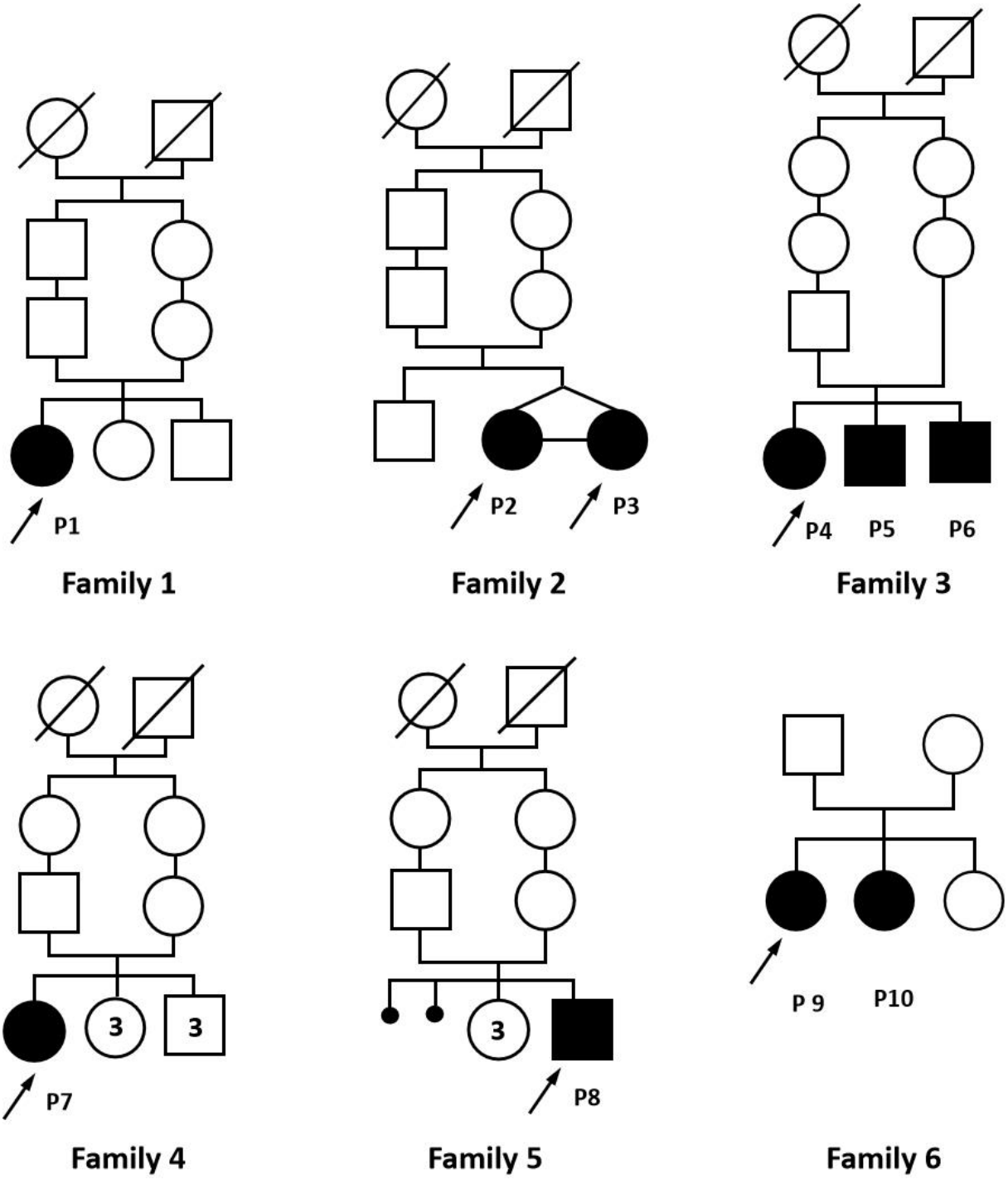

Figure 1: Pedigrees of six Tunisian families with PMM2-CDG.

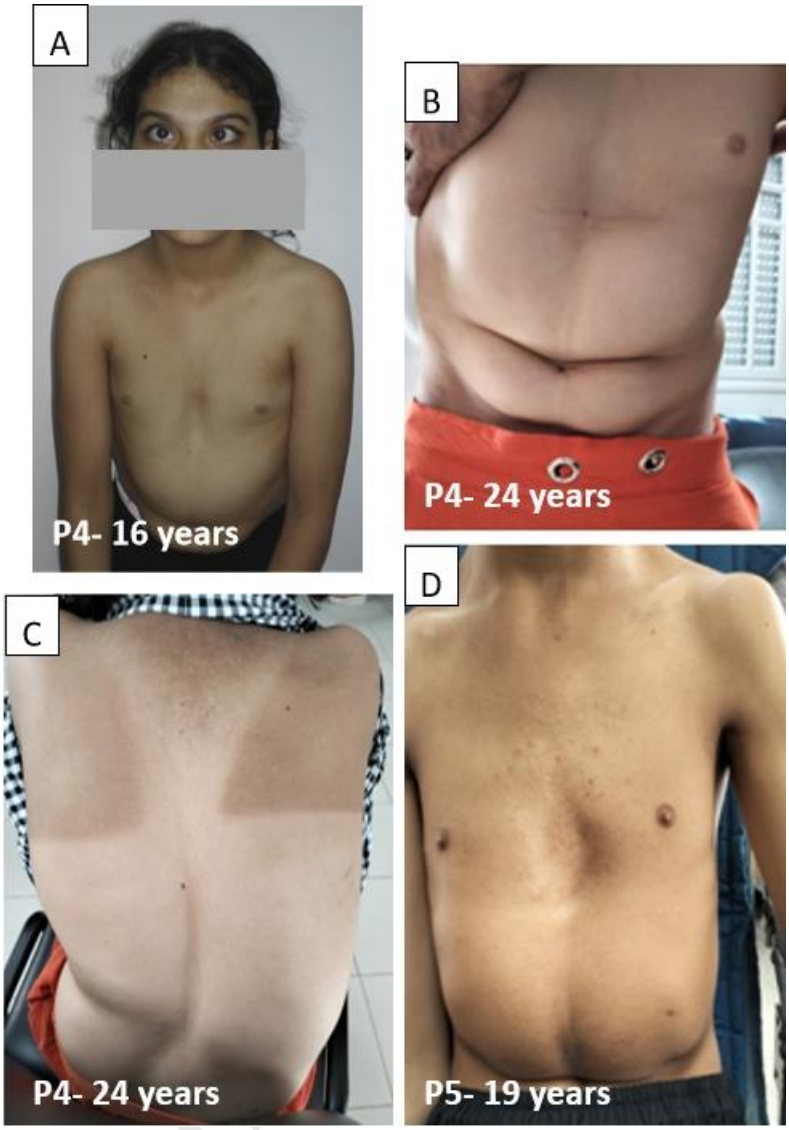

Figure 2: A 16-year-old patient (P4) diagnosed with PMM2-CDG presenting with bilateral convergent strabismus, pectus excavatum, and delayed puberty, evidenced by the absence of breast development **(A).** At age 24, the same patient exhibited abnormal fat distribution with a prominent suprapubic fat pad (B) and scoliosis (C). A 19-year-old patient (P5) showing pectus excavatum and an increased suprapubic fat deposition (D).

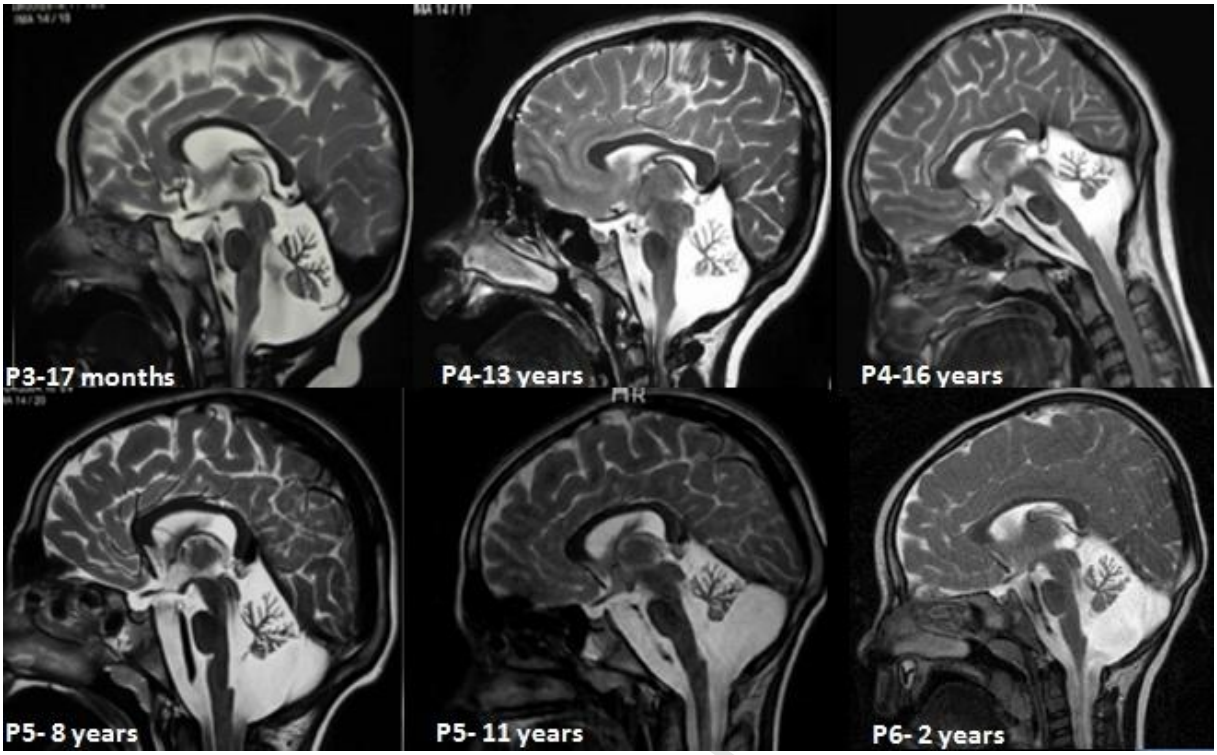

Figure 3: Sagittal T2-weighted brain MRI showing marked cerebellar atrophy involving both the vermis and hemispheres. No progression was observed on follow-up imaging after three years (P4 and P5).

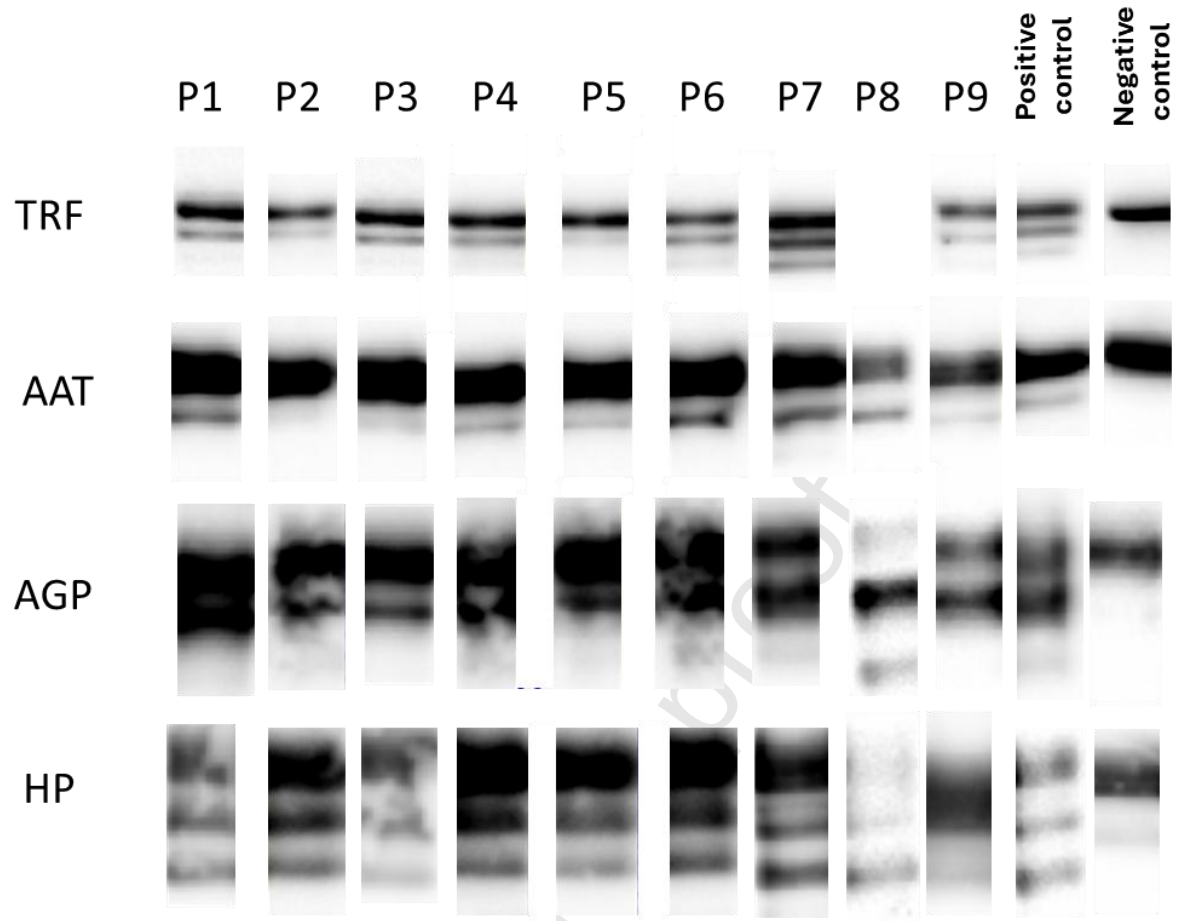

Figure 4: Western blot analysis of serum glycoproteins derived from patients P1-P9 (transferrin (TRF), alpha-1-antitrypsin (AAT), haptoglobin (HP), and alpha-1-acid glycoprotein (AGP)) showing bands of lower molecular weight compared to healthy controls, consistent with a diagnosis of type I CDG.

		Family 1¤	Family 2		Family 3			Family 4*	Family 5*	Family 6	
		P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
		M	F	F	F	M	M	F	M	F	F
st consultation		6y6m	14m	14m	13y	8y10m	2y	13y	17m	8y8m	12m
t assessment		16y	3y6m	3y6m	24y4m	19y6m	4y	15y3m	2y9m	13y	3y
ic origin: City/country		Kasserine/Tunisia	Kasserine/Tunisia		Bizerte/Tunisia			Kasserine/ Tunisia	Kasserine/Tunisia	Kef/Tunisia	
inity		+	+		+			+	+	- **	
ptoms	Psychomotor delay	+	+	+	+	+	+	+	+	+	+
	Hypotonia	-	-	-	-	-	-	+	+	+	+
	Gait ataxia	+	-	-	-	-	-	-	-	-	-
eatures	Cerebellar ataxia	+	+	+	+	+	+	+	+	+	-
	Strabismus	+	+	+	+	+	+	+	-	+	+
	Nystagmus	-	-	-	+	-	+	+	-	+	-
	Retinitis	-	-	-	NP	NP	NP	-	NP	-	NP
	Distal amyotrophy	+	-	-	+	+	+	-	-	-	-
	Diminished deep tendon reflexes	-	-	-	+	-	+	+	+	+	+
	Intellectual disability (severity)	+ (Mild to moderate)	NA	NA	+ (Moderate)	+ (Moderate)	NA	+ (Moderate)	NA	+ (Moderate, IQ: 50)	NA
	Stereotypic behavior	-	+	+	-	+	+	-	-	-	-
	Jovial mood	-	+	+	+	+	-	-	-	-	-
	Microcephaly	+ (-3SD)	+ (-3SD)	+ (-2SD)	+ (-3SD)	+ (-2SD)	+ (-3SD)	-	-	+ (-3SD)	+ (-2.5SD)
	Growth delay	-	-	-	+ (weight -2.6SD, height-3SD)	+ (height -3SD)	-	-	+ (Weight -3SD)	-	+ (Weight -4SD, height -3 SD)

	Delayed puberty	-	**NA**	**NA**	+	-	**NA**	+	**NA**	-	**NA**
	Dysmorphic facial features	+	+	+	+	+	+	+	+	-	+
	Inverted nipples	+	-	-	-	-	-	-	-	-	+
	Orange peel skin	-	-	-	+	+	-	+	-	-	-
	Abnormal fat distribution	-	-	-	+	+	-	+	-	-	-
	Pectus excavatum	+	-	-	+	+	+	-	-	-	-
	Scoliosis	-	-	-	+	-	-	+	-	-	-
	Kyphosis	-	-	-	-	+	-	-	-	-	-
	Flat feet	-	-	+	+	+	-	+	+	+	+
	Clinodactyly	-	-	-	+	+	+	+	+	+	+
RI	**Cerebellar atrophy**	+ **With Dandy-Walker variant**	+	+	+	+	+	+	+	+	+
	Pontine atrophy	-	-	-	-	-	-	+	-	-	-
e conduction velocities		**+ (12y)**	**NP**	**NP**	**+ (16y)**	**+ (11y)**	**+ (4y)**	**+ (15y)**	**NP**	**+ (9y)**	-
al/renal d	**Hepatomegaly**	-	-	+	-	-	-	-	-	-	**NP**
	Renal anomalies	-	**+ Renal hyperechogenicity and duplicated collecting system**	**+ Renal hyperechogenicity**	-	-	-	-	-	-	**NP**
iogram	**Cardiac anomalies**	-	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	-	-	**NP**
ry	**Cholesterol**	↘	**N**	**N**	↘	↘	↘	↘	**N**	↘	↘
	Transaminases	↗	↗	↗	**N**	**N**	↗	**N**	↗	**N**	↗
	LDH	↗	↗	↗	**N**	**N**	↗	**N**	↗	↗	↗
	Albumin	↘	**N**	**N**	**N**	**N**	**NP**	**NP**	**NP**	↘	**NP**

	CPK	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
	Thyroid test	**N**	**N**	**N**	**N**	**N**	**NP**	**N**	**N**	**N**	**N**
	IgA	**NP**	**↘**	**↘**	**↘**	**NP**	**NP**	**N**	**NP**	**N**	**NP**
	Hemoglobin	**N**	**↘**	**↘**	**N**	**N**	**N**	**↘**	**↘**	**N**	**N**
	PT	**N**	**N**	**N**	**N**	**NP**	**NP**	**↗**	**NP**	**N**	**NP**
	Anti-thrombin III	**↘**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**↘**
	Protein S	**↘**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**N**
	Protein C	**N**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**NP**	**↘**
PMM activity (U/g) g)		**↘0.8**	**↘0.6**	**↘0.9**	**↘0.8**	**↘0.9**	**↘0.7**	**NP**	**NP**	**NP**	**NP**
PMM activity (U/g) g) (Father/Mother)		**↘ 2,5/4,8**	**↘ NP/2,5**		**↘ 2,4/2,8**			**NP**	**NP**	**NP**	**NP**
***PMM2* sequencing 0303.3)**		**Homozygous variant in exon 5 of the *PMM2* gene: c.395T>C; p.(Ile132Thr) in all patients**									
***PMM2* sequencing 0303.3)**		**Heterozygous variant in exon 5 of the *PMM2* gene: c.395T>C; p.(Ile132Thr) in the parents*** **									

Table 1: Main clinical, radiological, laboratory, and genetic findings in Tunisian patients with PMM2-CDG.

P: Patient; F: Female; M: Male; m: Months; y: Years; IQ: Intelligence quotient; N: Normal; NA: Not applicable; NP: Not performed; NV: Normal Value; PMM: Phosphomannomutase; LDH: Lactate dehydrogenase; CPK: Creatine phosphokinase; TSH: Thyroid-stimulating hormone, IgA: Immunoglobulin A, PT : Prothrombin time; SD: Standard deviation; +: Present; -: Absent; ↘: Low level; ↗: High level; *: Identical surname with no established familial relationship;** : Endogamy ;***: The father of Family 2 and the father of Patient 8 were not tested.

Table 2: Haplotypes of six patients from six unrelated Tunisian families.

Alleles in bold represent those in linkage disequilibrium with the NM_000303.3(*PMM2*): c.395T>C; p.(Ile132Thr) variant. The six patients from six families shared the same polymorphic variants at each site tested, compared to three non-mutated Tunisian controls.

	Extragenic microsatellite markers of *PMM2* gene						
	D16S513	**12538882**	**12466423**	**D16S768**	**D16S3020**	**12457635**	**D16S406**
P1/F1	**185-185**	**108-108**	**159-159**	**132-132**	**85-85**	**135-135**	**194-194**
P2/F2	**185-185**	**108-108**	**159-159**	**132-132**	**85-85**	**135-135**	**194-194**
P4/F3	**185-185**	**108-108**	**159-159**	**132-132**	**85-85**	**135-135**	**194-194**
P7/F4	**185-185**	**108-108**	**159-159**	**132-132**	83-**85**	**135-135**	**194-194**
P8/F5	**185-185**	**108-108**	**159-159**	**132-132**	**85-85**	**135-135**	**194-194**
P9/F6	**185-185**	**108-108**	**159-159**	**132-132**	83-**85**	**135-135**	192-**194**
Control 1	**185-185**	**108-108**	155-155	**132-132**	89-89	131-131	184-184
Control 2	171-**185**	110-**108**	155-155	123-128	75-83	131-137	186-188
Control 3	171-**185**	104-**108**	155-155	**132-132**	73-75	131-137	182-186

P: Patient, F: Family

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Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

On behalf of all authors

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