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Clinical and Molecular Features of Patients with Congenital Disorders of Glycosylation in Brazil

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Abstract

Introduction: Congenital Disorders of Glycosylation are a group of genetic disorders due to abnormal glycosylation of glycoproteins and glycolipids. Based on isoelectric focusing of plasma transferrin results, CDG are classified in two groups: CDG-I and CDG-II. While the diagnosis of PMM2-CDG (formerly CDG-Ia) and PMI-CDG (formerly CDG-Ib) is made by demonstration of the enzyme deficiency or by gene sequencing, the diagnosis of the other CDG is not easily performed. Psychomotor delay/mental retardation, hypotonia, seizures, ataxia, cerebellar atrophy, strabismus, inverted nipples, lipodystrophy, and stroke-like episodes characterize PMM2-CDG, by far the most common CDG. There is almost no information available in the literature on the frequency of CDG in patients with psychomotor delay/mental retardation.

Patients and methods: We performed transferrin isoelectric focusing in 2619 patients who had psychomotor delay/mental retardation associated with other symptoms suggestive of CDG. Determination of leukocyte phosphomannomutase and phosphomannoseisomerase activities and *PMM2* gene sequencing was performed in selected patients.

Results: We found 32 affected patients (26 CDG-I and 6-CDG-II). CDG-I group: The most prevalent PMM2-CDG clinical symptoms were those expected. We identified two novel mutations: p.G79V and p.R21W. Non-PMM2, non-PMI-CDG showed more frequently coagulopathy, hypotonia, cerebellar atrophy, and cryptorchidism/micropenis. Early deaths were found exclusively in this group. Ataxia, strabismus, elevated blood FSH and LH levels were more frequent in PMM2-CDG patients. CDG-II group: four out of six patients presented *cutis laxa*, seizures, large fontanel, facial dysmorphism, and non-lissencephalic cortical dysplasia. Hip luxation was present in three patients, and hydronephrosis in one. The other two patients had heterogeneous features.

Conclusions: We determined the frequency of CDG in a selected Brazilian cohort with symptoms suggestive of CDG as 1.2%(CDG-I ~ 1.0% and CDG-II ~ 0.2%), and identified two novel mutations in the *PMM2* gene.

Keywords: CDG; Congenital disorders of glycosylation;Frequency; Glycosylation; Inborn errors of metabolism; Psychomotor delay; Mental retardation; Ataxia; Coagulation;Cutis laxa

Abbreviations: CDG: Congenital Disorders of Glycosylation; PMM2: Gene coding for the enzyme phosphomannomutase; PMI: Gene coding for phosphomannoseisomerase; FSH:Follicle Stimulant Hormone; LH:Luteinizing Hormone; Tf IEF:Isoelectric Focusing of Transferrin; PMD/MR: Psychomotor Delay/Mental Retardation; ALT:Alanine aminotransferase; AST:Aspartate aminotransferase; PCR: Polymerase Chain Reaction; CK:Creatine kinase

Introduction

Congenital Disorders of Glycosylation are pan-ethnic diseases [1,2] caused by the defective glycosylation of glycoproteins and glycolipids. Some 50 CDG have already been recognized [3]. Clinical manifestations range from severe multisystemic disease [4-7] to organ-specific involvement [8,9]. The standard method of screening for CDG is plasma Tf IEF. There are two abnormal patterns: CDG type I (defective synthesis and incorporation of the oligosaccharide side chain), and CDG type II (defects in the processing of the oligosaccharide side chain). Some 77-83% of CDG-I patients have deficient phophomannomutase activity (PMM2-CDG) [4,10]. PMM2-CDG has an estimated frequency of 1:20,000 births [11]. Except for PMI-CDG [12], and some patients with SLC35C1-CDG [13,14], there is no treatment available for CDG.

The general clinical picture of CDG is non-specific: PMD/MR, failure to thrive, seizures, ataxia, hypotonia, cerebellar atrophy, mild facial dysmorphism, strabismus, inverted nipples, hypogonadism, hepatopathy, peripheral neuropathy, coagulopathy, and stroke-like episodes. Due to the non-specificity of the clinical picture, several authors have recommended investigating CDG in patients with at least two affected organs or systems, especially when neurologic disorder (cerebellar hypoplasia, hypotonia, PMD/MR, and seizures), progressive ophtalmopathy or coagulopathy are present [7,8]. Others suggest to screen for CDG in any unexplained clinical disorder [15-18]. Apart from the unspecific clinical manifestations,these disorders are underdiagnosed for a number of reasons: medical unawareness of the disease, increased mortality in the first years of life, and unavailability

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of widespread specific laboratory tests. We were unable to find in the literature the frequency of CDG among children with symptoms suggestive of CDG.There is scarce information on Latin American patients with CDG [19-21].

This study aimed to determine the frequency of the disease in a large cohort of patients using broad inclusion criteria (PMD/MR associated with other symptoms suggestive of CDG), to study its clinical, radiologic, and laboratory characteristics, to determine the frequency of CDG-I and II, as well as to reveal the mutations in the *PMM2* gene among Brazilian patients.

Patients and Methods

A total of 2619 patients presenting PMD/MR associated with other symptoms suggestive of CDG (ataxia, seizures, stereotypic movements, hypotonia, macro/microcephaly, delay of myelination, cerebellar atrophy, stroke, encephalopathy, iris coloboma, retinitis pigmentosa, strabismus, craniofacial dysmorphism, lipodystrophy, inverted nipples, failure to thrive, hypogonadism, or ichthyosis) were submitted for plasma Tf IEF [22,23]. According to the abnormal Tf IEF pattern, the patients were classified as CDG-I or -II. The *PMM2* gene was sequenced in the CDG-I patients, and/or leukocyte PMM and PMI activities determined [10]. According to PMM and PMI activities, CDG-I patients were then subdivided in PMM2-CDG or non-PMM2, non-PMI-CDG-I groups.

Mutation analysis of the *PMM2* gene: Genomic DNA was extracted from blood samples anticoagulated with EDTA using standard procedures. The eight protein coding exons and flanking intronic sequences were directly sequenced after PCR [1,24]. Bidirectional sequencing was performed using the Big Dye Terminator Sequencing Kit (Applied Biosystems) according to the manufacturer's protocol, and analyzed with a 3130xl Genetic Analyzer (Applied Biosystems).

Clinical, radiological, laboratory data were both retrospectively and prospectively obtained from the patients' medical charts. Comparison of frequencies of clinical and laboratory findings between PMM2-CDG and non-PMM2, non-PMI-CDG-I groups were performed using Fisher's Exact Test (software SSPS v13.0). The research project was approved by the Research Ethical Committee at the SARAH Network of Rehabilitation Hospitals.

Coagulation factors VIII, IX, XI, antithrombin, protein C, and free protein S were measured with kits from DiagnosticaStago (Asnière, France) using standard procedures. Other routine biochemical tests were performed (hemogram, serum transaminases, glycemia, TSH and T4, renal function tests, blood gases, and inborn errors of metabolism investigation), but only the relevant data were included in this study.

Patients:	1	2	3*	4*	5	6	7	8	9	10*	11*	12	13	Total
Sex	F	F	F	F	F	F	M	M	F	F	F	M	M	
Age at diagnosis	2 y	3 y	23 y	21 y	1 y	18 y	4 y	1 y	1 y	23 y	26 y	6 y	2 y	
Present age	9 y	7 y	28 y	25 y	6 y	23 y	10 y	7 y	7 y	25 y	28 y	8 y	4 y	
PMM activity (control range)**	0.17 (0.96-3.56)	na	1.93 (1.20-3.79)	0.57 (1.20-3.79)	na	na	0.08 (2.09-5.43)	0.16 (2.09-5.43)	na	na	na	na	na	
PMM2 mutations	T226S R141H G79V	T237M R141H	T226S R141H	T226S R141H	R123X D223E	F119L R141H	T226S R21W	D65Y R141H	F157S R162W	T237M R141H	T237M R141H	T237MRR141H	T237M R141H	
Neurological signs														
Developmental delay / MR	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Ataxia	+	+	+	+	-	+	+	-	+	+	-	+	+	10/13
Hypotonia	-	+	-	-	+	-	+	+	+	+	+	+	+	9/13
Hyporeflexia	+	-	-	+	+	+	+	+	+	+	+	-	-	9/13
Strabismus	-	+	+	+	+	+	+	+	+	+	+	+	+	12/13
Cerebellar atrophy	+	+	+	+	+	+	+	+	+	+	+	+	-	12/13
Other clinical signs														
Short stature	+	-	+	-	+	+	na	-	+	+	na	na	+	7/10
Facial dysmorphism	+	-	+	+	+	+	-	+	+	+	+	-	+	10/13
Laboratory data														
Coagulopathy	+	+	+	-	+	+	+	na	+	+	+	+	na	10/11
Elevated FSH/LH	+	+	-	+	na	+	-	na	+	+	+	-	na	7/10

F: female; M:male; MR: mental retardation; y: year; +: present; -: absent; na: information not available. * Patients 3 and 4, and patients 10 and 11 represent pairs of sibs; ** PMM activities are expressed in mU/mg protein.

Table 1: Main clinical findings in PMM2-CDG patients.

Results

A total of 32 patients (1.2%; 14 males and 18 females) were identified. Based on the patterns found on Tf IEF (type I = 26 patients; type II = 6 patients), molecular studies, and/or enzyme determinations, they were classified into different groups:

CDG-I group (n=26)

Twenty-six patients presented Tf IEF type I pattern (1.0%; 26/2619). PMM2-CDG was confirmed in 13 individuals by demonstration of PMM deficiency and/or *PMM2* gene mutations. Eight patients had normal PMM and PMI activities, and were thus classified as non-PMM2, non-PMI-CDG I. Five individuals represent lost cases and were considered only for the calculation of CDG-I frequency.

PMM2-CDG patients: Clinical findings are summarized in table 1, and patients faces are shown in figure 1. Nine patients were females and four were males. Coagulopathy, strabismus and cerebellar atrophy was seen in almost all patients. All other clinical symptoms were consistently found. Patient 4 had isolated protein C value very close above the upper limit, and were considered as not having coagulopathy. Elevated FSH/LH levels were found in 7/8 females and in 0/2 males.

Non-PMM2, non-PMI-CDG-I patients: Clinical findings are summarized in table 2 and facial features are shown in figure 2. Four

Legend (figure 1): Dysmorphism can be subtle and are not homogeneous. Almond-shaped eyes in patients A, E, I, and L; bitemporal narrowing in patients A, E, F, and I, prominent chin in patients C, D, F, J, and K; inverted nipples in patients A and B. Figures A to M (patients 1 to 13; table 1).

Figure 1: Dysmorphism of PMM2-CDG patients.

Patient	1	2	3	4	5	6*	7*	8	Total
Sex	M	F	F	M	M	F	F	M	
Age at diagnosis	27 d	7 y	3 y	1 y	1 y	2 y	1 y	9 mo	
Present age	---	12 y	9 y	8 y	---	---	1 y	1 y	
Age of death	9 mo (?)				2y 9mo.	3y 9mo.			3/8
Developmental delay / MR	-	+	+	+	+	+	+	+	7/8
Hypotonia	-	+	+	+	+	+	+	+	7/8
Abnormal cerebral imaging	cerebellar atrophy + leukoencephalopathy	cerebellar atrophy + stroke	cerebellar atrophy	normal	cerebral atrophy	cerebellar atrophy	na	normal	5/7
Cryptorchidism / Micropenis	+	/	/	+	+	/	/	-	3/4
Elevated transaminases	na	+	+	+	+	-	-	+	5/7
Coagulopathy	na	+	+	+	+	+	+	+	7/7
Remarkable findings	Fetal hydrops		Glaucoma	Acanthosis nigricans				Ascites	

*Patients 6 and 7 are siblings; F: female; M:male; d: days; y: years; mo: months; MR: mental retardation; +: present; -: absent; /: data does not apply; na: information not available.

Table 2: Main clinical findings in non-PMM, non-PMI-CDG-I patients.

patients were females and four were males. Coagulation disorder was seen in all evaluated patients. PMD/MR and hypotonia was present in 7/8 patients. One patient was considered as not having PMD, because he was evaluated at a very young age (one month of age) and died before the next medical consultation. Abnormal cerebral imaging was detected in 5/7 patients (two boys had normal imaging). We found in this group, not only cerebellar atrophy, but also leukoencephalopathy, cerebral atrophy and stroke. Cryptorchidism/micropenis was found in 3/4 boys. Abnormal serum transaminases levels were seen in 5/7 patients. Three deaths occurred in this group (ages 9 months, 2 y 9 mo, and 3 y 9 mo).

Comparison between PMM2-CDG and non-PMM2, non-PMI-CDG-I patients: Most clinical features were equally seen in both groups, as determined by statistical analysis. Statistically significant differences ($p<0.05$) were limited to ataxia ($p=0.032$), strabismus ($p=0.047$), and elevated FSH and LH ($p=0.026$), which predominated among PMM2-CDG patients, while deaths occurred exclusively among non-PMM2, non-PMI-CDG-I patients ($p=0.042$). This latter group showed also higher frequency of abnormal results for antithrombin ($p=0.035$) and protein S ($p=0.013$), when compared to PMM2-CDG patients (tables 3 and 4). Stroke was found only in one non-PMM2, non-PMI-CDG-I patient.

CDG-II group (n=6)

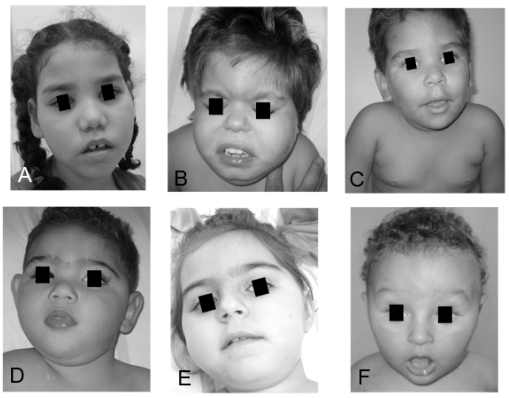
A total of 6 patients with CDG II pattern in Tf IEF (0.2%; 6/2619) were identified in this study. All presented coagulopathy. As shown in figure 3 and in table 5, four patients had similar clinical features: *cutis laxa*, dysmorphism, large fontanel, non-lissencephalic cortical dysplasia (figure 4), and low levels of free protein S. Except for one, all presented also hip luxation and seizures. Low levels of factor XI were seen in three. The other two patients had dysmorphism: one presented also failure to thrive, cataract, myopathy and increased level of CK, and factor VIII. The other individual had macrocephahly, obesity, strabismus, myopia and increased levels of factor XI and protein C.

PMM2 gene

R141H was the most prevalent mutation, present in 10/13 patients. One of these patients had also two other mutations: a novel (p.G79V; c.236G>T) and a common one (p.T226S). Other frequent mutations were p.T237M (n=5) and p.T226S (n=4). Interestingly, the former was found only in patients from Greater Belo Horizonte City area. One patient had another novel mutation (p.R21W; c.61C>T).

Discussion

There is scarce data in the literature on frequency of CDG in populations. Schollen et al. [11] estimated the frequency of PMM2-



Legend (figure 2): Facial features with striking variability (from mucopolysaccharidosis-like face in patient B to almost normal face in patient C). Figures A to E (patients 2 to 6; table 2); F (patient 8; table 2).

Figure 2: Facial features of non-PMM2, non-PMI-CDG patients.

Patients:	1	2	3	4	5	6	7	9	10	11	12	Abnormal results
PT (RNI;1.0-1.2)	1.0	ND	1.0	1.0	ND	1.0	1.0	1.1	ND	90% (70-120)	ND	0/8
aPTT (Pt/Cnt; <1.3)	**1.53**	ND	1.1	1.2	ND	1.0	1.1	0.9	ND	1.0	ND	1/8
Factor VIII (60-150%)	**>200**	**>200**	126	92	**>200**	**>200**	**>200**	**168**	**33**	108	67	7/11
Factor XI (60-140%)	**20**	**58**	79	83	**43**	**29**	**53**	78	110	94	**53**	6/11
Antithrombin (80-120%)	**37**	**79**	74	91	**30**	ND	**76**	100	102	**60**	109	4/10
Protein C (70-130%)	**34**	100	112	**131**	**32 †**	**86**	71	104	97	80	90	4/11
Protein S (70-130%)	**49**	70	**65**	70	**51ǂ**	**62**	**65**	74	**55 ‡**	**60 ‡**	79ǂ	7/11

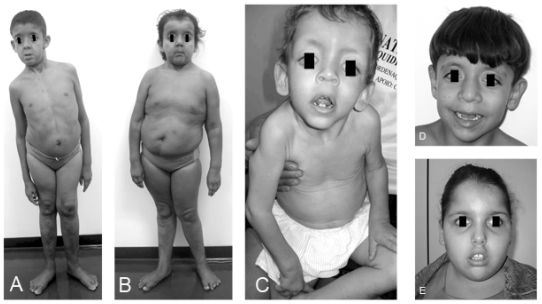
Normal values between parenthesis; Abnormal values are in bold.
Patients 8 and 13 had no coagulation tests performed. All patients had normal results for factor IX.
aPTT: activated partial tromboplastin time; PT: protrombin time; Pt: patient; Cnt: control; ND:not done; reference ranges †: 70-120%; ‡ : 64-126%; ǂ : 72-150%.

Table 3: Plasma levels of prothrombin time, activated partial thromboplastin time, coagulation factors, and anticlotting factors of PMM2-CDG patients.

Patients:	2	3	4	5	6*	7*	8	Abnormal results
Factor VIII (60-150%)	**>200**	101	**44**	**>200**	**175**	122	65	4/7
Factor IX (60-150%)	74	63	**40**	140	78	108	71	1/7
Factor XI (60-140%)	**11**	**28**	**12**	**21**	**49**	77	**10**	6/7
Antithrombin (80-120%)	**28**	**55**	**39**	**37**	**31**	**60**	**40**	7/7
Protein C (70-130%)	**34 †**	**58**	**48**	**59**	**56 †**	**45 †**	**24**	7/7
Protein S (70-130%)	**48 †**	**68**	**58**	**46╪**	**60 ‡**	**61 ‡**	**42 ╪**	7/7
aPTT (Patient/Control; <1.3)	37 seconds (25-38)	1.0	**1.3**	**1.3**	ND	ND	ND	2/4
PT (IRN;1.0-1.2)	**1,6**	1.0	1.0	1.1	ND	ND	ND	¼

* Siblings.
Normal values between parenthesis; Abnormal values are in bold.
Patient 1 had no coagulation tests performed.
aPTT: activated partial thromboplastin time; PT: prothrombin time; INR: International Normalized Ratio; ND=not done; reference ranges † =70-120%; ‡ = 64-126%; ╪ = 72-150%.

Table 4: Plasma levels of coagulation factors, natural anticoagulants, activated partial thromboplastin time, and prothrombin time of non-PMM2, non-PMI-CDG-I patients.



Legend (figure 3): Patients A, B and C share the same features: down-slanting palpebral fissures, cutis laxa, progeroid aspect, and scoliosis. Patient D has only down-slanting palpebral fissures. Patient E shows bitemporal narrowing, small nose with short philtrum. Figures A and B (patients 1 and 2; table 4); C (patient 4; table 4), D and E (patients 5 and 6; table 4).

Figure 3: Features of CDG II patients.

Patient	Consanguinity	Age at diagnosis	Present age	Gender	Clinical	Radiologic	Laboratory
1	-	9 y	12	Male	Mental retardation Cutis laxa Seizures Dysmorphism Large fontanel Myopia	Non-lissencephalic cortical dysplasia Hip luxation	Protein S= 54% (72-150) Tf IEF: ↑ 3S, 2S
2	-	6 y	8	Female	Mental retardation Cutis laxa Seizures Dysmorphism Large fontanel Myopia	Non-lissencephalic cortical dysplasia Hip luxation Scoliosis Genu valgum	Protein S= 51% (64-126) Factor XI= 34% (60-140) Tf IEF: ↑ 3S, 2S
3	? (adopted)	6 y	12	Female	Mental retardation Cutis laxa Seizures Dysmorphism Large fontanel	Non-lissencephalic cortical dysplasia Hip luxation Hydronephrosis	Protein S= 61% (64-126) Factor XI= 52% (60-140) Antithrombin= 68% (80-120) Tf IEF: ↑ 3S, 2S, 1S
4	+	1 y	3	Male	Mental retardation Cutis laxa Dysmorphism Large fontanel Microcephaly Unilateral chorioretinitis	Non-lissencephalic cortical dysplasia Hydronephrosis	Protein S= 55% (72-150) Factor VIII >200% (60-150) Factor XI= 54% (60-140) Factor VIII >200% (60-150) Tf IEF: ↑ 3S, 2S
5	+ (adopted)	5 y	10	Male	Mental retardation Dysmorphism Failure to thrive Cataract	Scoliosis	ENMG: myopathy Protein S= 47% (75-141) Factor VIII = 180% (60-150) CK= 241-996 IU/L (24-190) Tf IEF: ↑ 3S, 2S, 1S
6	-	6 y	14	Female	Mental retardation Dysmorphism Macrocephaly Obesity Myopia / strabismus	Flatfoot	Factor VIII >200% (60-150) Factor IX >200% (60-150) Factor XI= 170% (60-140) Tf IEF: ↑ 3S

+: present; -: absent; y: years; ENMG: electroneuromyography; Tf IEF: isoelectric focusing of transferrin; 3S: trisialotranferrin; 2S: disialotransferrin; monosialotransferrin. Normal values for clotting and anticlotting factors are between parenthesis.

Table 5: Main clinical findings in CDG-II patients.

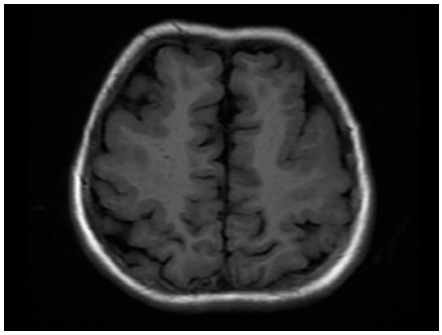


Figure 4: Polymicrogyria/Pachygyriacomplex (CDG II patient 2).

CDG as 1:20,000 births, and found no increase of the carrier frequency for the R141H mutation in a cohort of 600 patients with mental retardation. Pérez-Cerdá et al. [25] found 50 CDG patients in a group of 7910 pediatric patients with clinical suspicion of metabolic diseases. This represents a frequency of 0.6%.

We used in the present study PMD/MR, the most prevalent symptom of the disease, as obligatory criterion associated with other symptom suggestive of CDG, in order to make the investigation directed to CDG symptoms.Using at least two symptoms as the inclusion criteria, we intended to increase the frequency of diagnosed cases, and thus, make clinical selection more effective.This would spare resources.

The frequency of the disease among our patients was relatively high (1.2%; CDG-I 1.0%; CDG-II 0.2%). PMM2-CDG was present in at least 0.5% (13/2619) of the patients, and non-PMM2, non-PMI-CDG-I in at least 0.3% (8/2619).

There is a general agreement between our findings and cohorts with neurologic forms of PMM2-CDG [4,26,27]. The frequency of PMM2-CDG (13/21; 62%) in our sample was similar to that reported in the literature (77-83% of CDG-I) [4,10]. PMD/MR, cerebellar atrophy, strabismus, coagulopathy, hypotonia, hyporeflexia, facial dysmorphism, short stature, and hypergonadotropichypogonadism (in females) were present in at least 69% of the PMM2-CDG patients. The presence of ataxia, strabismus and hypergonadotropichypogonadism (in females), although not exclusively seen in PMM2-CDG, should favor this diagnosis. We identified 11 mutations in the *PMM2* gene. The allele p.R141H is present in 47-81% of PMM2-CDG families in Europe [4,26,28]. In our study this allele was found in 8/11 families (73%). Two previously unreported mutations in the *PMM2* gene were found. The p.R21W mutation was considered to be pathogenic. In the *PMM1* gene Arg28 is the sole core domain contact, which interacts with the C-4 hydroxyl group of the substrate. This amino acid is located in the position 21 in the *PMM2* gene [29]. Its substitution for trytptophanwill prevent binding to the substrate. Patient 7, who has this novel mutation and the known p.T226S, shows enzyme activity close to zero. The p.G79V mutation was associated with two known pathogenic mutations (p.R141H and p.T226S). We submitted this mutation to algorithms (SNPs3D and nsSNPAnalyzer)that predict pathogenicity [30,31]. Both algorithms predicted p.G79V to be pathogenic. As shown in Table 1, this additional mutation seems to afffect significantly the protein function, as compared with other patients (patients 3 and 4). The mutation p.T237M was found exclusively in patients from Belo Horizonte city area and is likely to represent a founder effect. Brazilian population's ancestry is composed largely by Portuguese and Italians immigrants. The mutation p.T237M has been identified among Italians [28]. Interestingly, all these patients bear Portuguese surnames. Hence, it could represent either a mutation in Portuguese genes or reveal an unknown Italian ancestry. The mutation p.D223E found in one family in the present study had only been identified among Danish [28]. The other mutations found in our study reveal the Iberic ancestry of Brazilian population. We could not find a genotype-phenotype correlation.

The most frequent clinical findings in our non-PMM2, non-PMI-CDG-I patients, such as PMD, hypotonia, liver involvement, cerebellar atrophy, and coagulopathy, are similar to those previously reported [32]. In our series, they did not show distinctive symptoms from PMM2-CDG, except for the higher frequency of death, probably because they represent more severe subtypes. Laboratory data suggestive of hypergonadotropichypogonadism were not found among these patients.

Clotting factors and anticlotting factors are glycoproteins. As many as 80% of the patients present coagulation abnormalities, but it seems that cerebral infarction is rare [33,34].Coagulopathy, defined by abnormal levels of anticlotting and clotting factors, was present in 16 of the 17 CDG-I patients (Tables 1 and 2), but cerebral infarction was seen in only one non-PMM2, non-PMI-CDG-I patient. Mechanisms underlying clotting disorders in CDG are complex and not fully understood. In our study, non-PMM2, non-PMI-CDG-I patients showed, in general, more severe deficiency of clotting and anticlotting factors. There was no correlation between the coagulopathy and the degree of neurologic involvement, except for the patient 2, who had cerebral infarction. It is important to notice that levels of abnormal glycosylated glycoproteins fluctuate with age, are likely to worsen with fever or other stresses [35], and represent solely a static photography at the moment of the testing. So, direct inference from glycoprotein levels might be misleading.

All CDG-II patients presented coagulopathy. Mental retardation, *cutis laxa*, facial dysmorphysm (down-slanting palpebral fissures, and porsteriorly rotated ears), and cerebral malformation were found in four patients with CDG-II, and probably represent a single entity. These features are consistent with the diagnosis of ATP6V0A2-CDG [36-38]. Myopathy is an uncommon feature in CDG-II and was reported in a patient with B4GALT1-CDG (formerly CDG IId) [39]. Similarly to this reported patient, CDG-II patient number 5 showed electroneuromyographic data suggestive of myopathy, elevated plasma CK, and asialo profile of Tf IEF (increased mono- to trisialotransferrin). We speculate that this is likely to be the diagnosis of this patient.

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