Congenital Disorders of Glycosylation in Portugal—Two Decades of Experience

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Objective To describe the clinical, biochemical, and genetic features of both new and previously reported patients with congenital disorders of glycosylation (CDGs) diagnosed in Portugal over the last 20 years.

Study design The cohort includes patients with an unexplained multisystem or single organ involvement, with or without psychomotor disability. Serum sialotransferrin isoforms and, whenever necessary, apolipoprotein CIII isoforms and glycan structures were analyzed. Additional studies included measurement of phosphomannomutase (PMM) activity and analysis of lipid-linked oligosaccharides in fibroblasts. Sanger sequencing and massive parallel sequencing were used to identify causal variants or the affected gene, respectively.

Results Sixty-three individuals were diagnosed covering 14 distinct CDGs; 43 patients diagnosed postnatally revealed a type 1, 14 a type 2, and 2 a normal pattern on serum transferrin isoelectrofocusing. The latter patients were identified by whole exome sequencing. Nine of them presented also a hypoglycosylation pattern on apolipoprotein CIII isoelectrofocusing, pointing to an associated O-glycosylation defect. Most of the patients (62%) are PMM2-CDG and the remaining carry pathogenic variants in *ALG1, ATP6AP1, ATP6AP2, ATP6V0A2, CCDC115, COG1, COG4, DPAGT1, MAN1B1, SLC35A2*, *SRD5A3*, *RFT1*, or *PGM1*.

Conclusions Portuguese patients with CDGs are presented in this report, some of them showing unique clinical phenotypes. Among the 14 genes mutated in Portuguese individuals, 8 are shared with a previously reported Spanish cohort. However, regarding the mutational spectrum of PMM2-CDG, the most frequent CDG, a striking similarity between the 2 populations was found, as only 1 mutated allele found in the Portuguese group has not been reported in Spain. *(J Pediatr 2021;231:148-56)*.

Because glycosylation occurs in all cells of the organism and approximately one-half of all proteins are glycoproteins,[1] it is not surprising that the congenital disorders of glycosylation (CDGs) show highly diverse clinical presentations. Currently, they comprise over 130 genetic diseases caused by any impairment in the assembly and attachment of glycoprotein and glycolipid glycans. There are 2 main categories of glycosylation pathways, the N-glycosylation and the O-glycosylation. N-glycans are linked to the amide group of asparagine, and O-glycans are linked to the hydroxyl group of serine or threonine of the protein moiety. The synthesis of N-glycans proceeds in 3 stages: formation of nucleotide-linked sugars in the cytosol, assembly in the cytosol and the endoplasmic reticulum, and processing in the Golgi. The synthesis of O-glycans does not involve processing and occurs mainly in the Golgi. Besides, there are also defects in lipid glycosylation and glycosylphosphatidylinositol anchor biosynthesis.[2]

The standard test for the diagnosis of N-glycosylation disorders with sialic acid deficiency is still isoelectrofocusing of serum transferrin, a liver synthesized

CDG	Congenital disorder of glycosylation
CDT	carbohydrate-deficient transferrin
PMM2	Phosphomannomutase 2

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Supported by National Funds through the Fundação para a Ciência e a Tecnologia, Portugal (Portuguese national funding agency for science, research and technology) in the frameworks of the UID/Multi/00215/2013 project–Unit for Multidisciplinary Research in Biomedicine–UMIB/ICB AS/UP. The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2020.12.026

N-glycosylated protein. A type 1 pattern (decreased tetrasialotransferrin, increased disialo- and asialotransferrin) points to a glycan assembly defect or a defect in the transfer of the glycan to the peptide chain (CDG-I), and a type 2 pattern (increase also of threesialo- and monosialotransferrin) suggests a remodeling defect (CDG-II).[2]

Approximately 60 genetic defects have been described in the N-glycosylation pathway, either isolated or in combination with O-glycosylation defects. Phosphomannomutase 2 (PMM2)-CDG is the most common CDG and still underdiagnosed.[3] Signs and symptoms of a CDG can appear at any age, but usually come to medical attention during infancy. The wide range of clinical presentations and severity of the symptoms, which often involve neurologic manifestations, justifies the need to screen for CDG-related genes in any unexplained disorder with a multisystem phenotype or even when only a single organ is involved. Clinical manifestations include involvement of the nervous system (global developmental disability, seizures, ataxia, hyporeflexia, polyneuropathy, olivopontocerebellar hypoplasia), musculature (generalized hypotonia, muscle weakness), cardiovascular system (cardiomyopathy, pericardial effusion), digestive system (feeding difficulties in infancy, hepatomegaly, elevated serum transaminases, hepatic fibrosis, steatosis), skeletal system (kyphosis, osteopenia), abnormalities of the eyes (nystagmus, strabismus, esotropia, retinopathy), head and neck (microcephaly, facial dysmorphism), skin (abnormal subcutaneous fat tissue distribution, cutis laxa, ichthyosis), endocrine system (hypergonadotropic hypogonadism, hyperinsulinism), immune system (immunoglobulin deficiencies, B and T cell disorders), blood and blood-forming tissues (dyserythropoietic anemia, thrombocytopenia, low coagulation factors), and growth (failure to thrive). Nonimmune *hydrops fetalis* can be a predominant manifestation in the perinatal period.

We present the features of 63 Portuguese patients with a confirmed molecular diagnosis during the last 20 years.

Methods

Most of the patients presented in this article were followed in hospitals from the Portuguese National Health Service and had mainly a multisystem disease of unknown etiology. These patients were selected on a clinical suspicion of CDG (particularly developmental/intellectual disability and liver disease) and/or the finding of laboratory abnormalities such as persistently elevated serum transaminases, coagulation factor decreases (decrease of antithrombin activity, factor XI, protein C, and protein S concentrations), hypoglycemia, hypoproteinemia, hypo- or hypercholesterolemia, decreased thyroxin-binding globulin/total thyroxine, and hyperinsulinism. Ferreira et al reported the recognizable phenotypes in CDG (n = 36), and Francisco et al enumerated the clinical features suggestive of (distinct CDG (n = 73, thus, approximately one-half of the known CDG number of

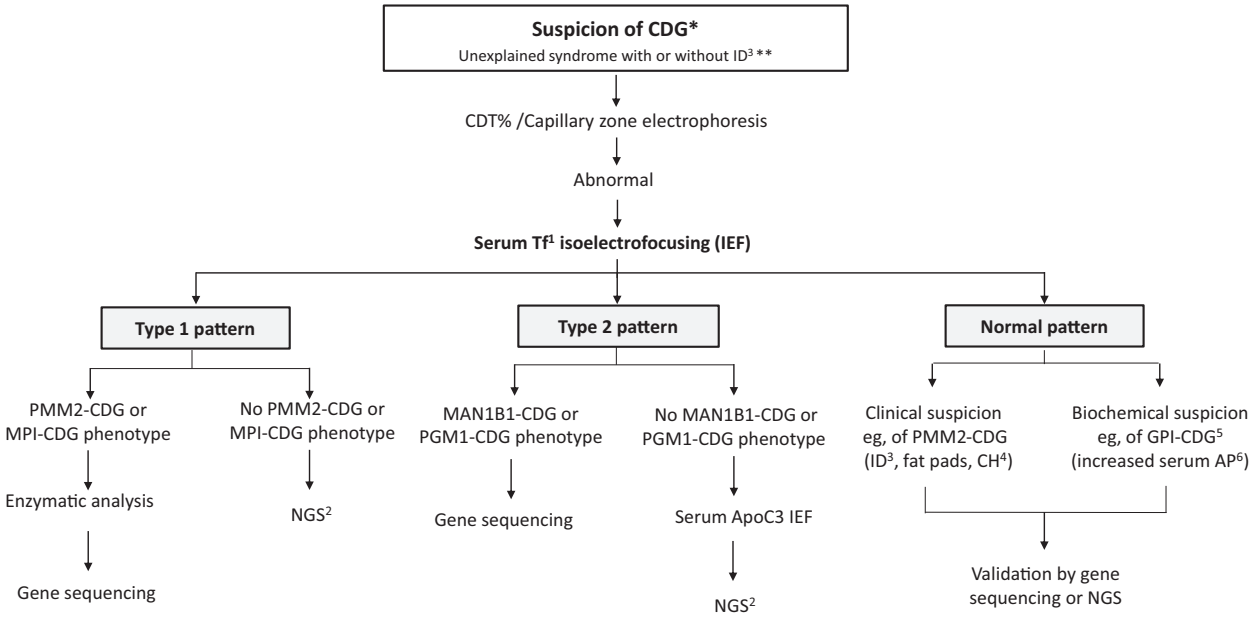

Figure. CDG diagnosis algorithm. (1) Transferrin; (2) Next generation sequencing (CDG panel analysis, WES, WGS); (3) Intellectual disability; (4) Cerebellar hypoplasia; (5) Glycosylphosphatidylinositol anchor synthesis defect; (6) Alkaline phosphatase. *Adapted from Francisco et al.[5] **Ferreira et al[4] and Francisco et al.[5] *ID*, intellectual disability; *WES*, whole exome sequencing; *WGS*, whole genome sequencing.

Table I. Core data of the patients (HPO designations used)

CDG phenotype MIM nr	Number of patients; families	Sex F;M	Age at diagnosis	Affected glycosylation pathway (serum transferrin IEF pattern)	Detection method	Major clinical signs and symptoms	Results of laboratory tests, histology and brain imaging
Defects in the cytosol							
PMM2-CDG #212065	39 (35 families)[13-18]	16 F; 19 M, 4 PND	birth to 19 y	N (1)	38 Sanger; 1 WES	Nonimmune hydrops fetalis Microcephaly, abnormal facial shape, mild to severe global developmental delay, ataxia, muscular hypotonia, dystonia Abnormal eye movements, strabismus Abnormal subcutaneous fat tissue distribution, generalized oedema, cryptorchidism, inverted nipples Feeding difficulties, vomiting and diarrhoea in infancy Hypertrophic cardiomyopathy, ventricular hypertrophy, pericardial effusion Abnormality of the skeletal system, arachnodactyly	Hypoalbuminemia, proteinuria, elevated serum transaminases Reduced protein C and protein S activity MRI: olivopontocerebellar hypoplasia
PGM1 # 614921	1	M	10 y	N (2)	Rhabdomyolysis gene panel	Hepatomegaly Submucous cleft hard palate Recurrent rhabdomyolysis associated with acute infections	Elevated serum transaminases, (asymptomatic) hypoglycaemia Elevated CK levels,
Defects in the ER							
SRD5A3-CDG # 612379	2 (1 family)[19]	1 F;1 M	8 y;14 y	N (1)	Sanger	Severe global developmental delay and intellectual disability, seizures, hyperreflexia Rod-cone dystrophy, nystagmus, alternating exotropia, strabismus and blue sclerae Short nose and gingival overgrowth, wide intermamillary distance and gynecomastia Abnormal subcutaneous fat tissue distribution and hirsutism	LLO: relatively low incorporation of radioactivity without specific changes Brain CT scan: normal
DPAGT1-CDG # 608093	2 (2 families)[20]	1 F;1 M	16 y; 5 y	N and O (1)	CDG and metabolic myopathies capture gene panel	Global developmental delay and intellectual disability, developmental regression, generalized myoclonic and tonic-clonic seizures, congenital muscular torticollis Myopathy, limb-girdle muscle weakness, fatigable weakness, proximal and distal amyotrophy, abnormal pyramidal sign and inability to walk Limited extraocular movements, ophthalmoparesis Inverted nipples, abnormal subcutaneous fat tissue distribution	Muscle biopsy: autophagic vacuoles, muscle fibre cytoplasmic inclusion bodies, muscle fibre tubular inclusions, type 1 muscle fiber predominance and decreased activity of mitochondrial respiratory chain MRI: global brain atrophy
ALG1-CDG # 608540	1[21]	1 M	7 y	N (1)	Sanger	Severe global developmental delay, microcephaly, seizures, generalized hypotonia	MRI: cerebellar atrophy
RFT1-CDG # 612015	3 (3 families)[22]	1 F;2 M	4 y;20 y; 2 y	N (1)	CDG capture gene panel	Intrauterine growth retardation Global developmental delay and intellectual disability (borderline to severe), seizures (1/3), sensorineural hearing impairment (1/3), ataxia, stereotypy (1/3), muscular hypotonia Abnormal facial shape with prominent forehead, deeply set eyes, epicanthus Scoliosis	Increased thyroid-stimulating hormone level, normal serum free T3 and T4 levels Prolonged partial thromboplastin time and prothrombin time Brain MRI: normal

(*continued*)

Table I. Continued

CDG phenotype MIM nr	Number of patients; families	Sex F;M	Age at diagnosis	Affected glycosylation pathway (serum transferrin IEF pattern)	Detection method	Major clinical signs and symptoms	Results of laboratory tests, histology and brain imaging
Defects in the Golgi/vacuolar membranes/vesicular membranes							
MAN1B1- CDG * 604346 # 614202	4 (4 families) [23]	1 F; 3M	7 y; 5 y; 7 y; 4 y	N (2)	Sanger; WES	Global developmental delay, intellectual disability, moderate to severe, macrocephaly, generalized hypotonia Long face, macrotia, low-set ears, epicanthus, downslanted palpebral fissures, hypertelorism, prominent nose, thin upper lip vermilion, short philtrum Retinal coloboma Long slender fingers Inverted nipples, wide intermamillary distance Truncal obesity Aggressive behaviour and stereotypy	Echography: aortic aneurysm MRI: normal (1/4) or cerebellar hypoplasia, cerebellar vermis hypoplasia
ATP6V0A2-CDG # 219200	2 (2 families) [24,25]	2 M	14 y; 3 mo	N and O (2)	Sanger; CDG capture gene panel	Mild to severe global developmental delay, Normal to moderate intellectual disability, seizures Failure to thrive Cutis laxa, redundant skin, abnormality of connective tissue Delayed cranial suture closure, large fontanelles, abnormality of the face, dermatochalasis, downslanted palpebral fissures, microretrognathia, high palate, abnormal location of ears and abnormality of the pinna Strabismus and corneal dystrophy (requiring transplantation) Lumbar hyperlordosis, thoracic kyphosis, genu recurvatum, joint hypermobility, bilateral congenital hip dislocation Multiple carious teeth Splenomegaly	Elevated serum aspartate aminotransferase Leukopenia, thrombocytopenia Reduced factor XI activity, prolonged prothrombin time, reduced protein C and protein S activity MRI: pachygyria, cerebellar vermis hypoplasia, Dandy-Walker malformation
ATP6AP1-CDG # 300972	1	1 M	1.5 y	N and O (2)	Sanger	No intellectual disability Relative macrocephaly, facial dysmorphism Pectus excavatum, scoliosis, joint hypermobility Hepatosplenomegaly Cryptorchidism Recurrent respiratory infections	Decreased circulating IgG and total IgM, hyperphosphatasia, hypercholesterolemia, decreased serum ceruloplasmin Liver biopsy: microvesicular hepatic steatosis, cirrhosis

(*continued*)

Table I. Continued

CDG phenotype MIM nr	Number of patients; families	Sex F;M	Age at diagnosis	Affected glycosylation pathway (serum transferrin IEF pattern)	Detection method	Major clinical signs and symptoms	Results of laboratory tests, histology and brain imaging
ATP6AP2-CDG * 300556 No MIM for the CDG phenotype	1 [26,27]	1 M	5 mo	N and O (2)	WES	Mild cognitive impairment Abnormal facial shape Prolonged neonatal jaundice, persistent hepatosplenomegaly and ascites Recurrent severe infections (eg, sepsis, peritonitis)	Leukocytosis, neutrophilia Elevated serum transaminases, hypoalbuminemia Prolonged partial thromboplastin time and prothrombin time Decreased antibody levels in blood, (IgG, IgM, IgA), specific anti-polysaccharide antibody deficiency, decreased proportion of CD4-positive T cells and CD8-positive T cells IgG hypoglycosylation Liver biopsy: macrovesicular hepatic steatosis, micronodular cirrhosis
COG1-CDG # 611209	1 [28]	1 F	7 mo	N and O (2)	Sanger	Mild psychomotor disability, progressive microcephaly Generalized hypotonia, mainly in upper limbs, but with normal muscle strength Narrow forehead and downslanted palpebral fissures, small hands and feet Feeding difficulties since birth and failure to thrive, no hepatosplenomegaly in infancy Left ventricular hypertrophy with left ventricular diastolic dysfunction After infancy: growth delay with rhizomelia and mild hepatosplenomegaly	MRI: slight cerebral and cerebellar hypoplasia
COG4-CDG # 613489	1 [29]	1 M	1 y	N and O (2)	Sanger	Moderate psychomotor disability, postnatal microcephaly, complex seizures, mild ataxia, axial hypotonia, slight peripheral hypertonia, hyperreflexia, brisk reflexes, limb dysmetria and poor speech. Abnormality of the face (down sloping frontal area and thick hair)	Elevated serum transaminases and alkaline phosphatase, increased lactate dehydrogenase activity, hypercholesterolemia Leukocytosis, thrombocytopenia Brain MRI: normal
CCDC115-CDG # 616828	1 [30]	1 F	5 mo	N and O (2)	WES	Generalized moderate hypotonia, with reduced muscle size Redundant skin Failure to thrive, jaundice, hepatosplenomegaly and progressive cholestatic liver disease Postmortem liver: severe cholestasis with intrahepatic portal vein sclerosis and cirrhosis	Hypoglycemia, hyperammonemia, elevated serum transaminases, alkaline phosphatase and total and LDL cholesterol Reduced factor VII activity, increased factor VIII activity Anemia with acanthocytes and reticulocytosis Bone marrow biopsy: hypoplastic anemia, some foam cells and hemophagocytosis Control biopsy after 2 months: bone-marrow foam cells and erythroid hypoplasia with perinuclear deposition of iron

(*continued*)

Table I. Continued

CDG phenotype MIM nr	Number of patients; families	Sex F;M	Age at diagnosis	Affected glycosylation pathway (serum transferrin IEF pattern)	Detection method	Major clinical signs and symptoms	Results of laboratory tests, histology and brain imaging
SLC35A2-CDG # 300896	4 (4 families) [31,32]	2 F;2 M	5 mo; 2 y; 3 y; 4 y	N and O (2)	CDG capture gene panel	Two distinct phenotypes: 1 without epilepsy Minor neurological involvement Growth deficiency associated with decreased serum IGF1 Minor facial dysmorphism, camptodactyly of fingers and toes 3 with epileptic encephalopathy Severe global developmental delay and intellectual disability Muscular hypotonia of the trunk, limb hypertonia Facial dysmorphism Yellow/white lesions of the retina, chorioretinal hyperpigmentation Hepatomegaly	Elevated serum- transaminases (aspartate aminotransferase >alanine aminotransferase) EEG: burst suppression and hypsarrhythmia Echo: left ventricular hypertrophy, abnormal atrial septum morphology Brain MRI: normal

ER, endoplasmic reticulum; *F*, female, *HPO*, human phenotype ontology; *LLO*, lipid-linked oligosaccharide; *MRI*, magnetic resonance imaging; *M*, male; *N*, N-glycosylation pathway; *O*, O-glycosylation pathway; *PND*, prenatal diagnosis; *T4*, total thyroxine.

137). The conclusion is that CDG should be considered in any unexplained syndrome, with or without intellectual disability.[4,5]

Data from major clinical signs and symptoms and diagnostic tests were collected by the referring clinicians.

The **Figure** is a graphic representation of the main diagnostic steps in CDG. The percentage of carbohydrate-deficient serum transferrin (sum of a-, mono-, and di-sialo transferrin isoforms; %carbohydrate-deficient transferrin [CDT]) was calculated according to %CDT Bio-Rad high performance (pressure) liquid chromatography protocol. When the percentage of the hypoglycosylated isoforms was abnormal, serum transferrin isoelectrofocusing[6] and/or capillary zone electrophoresis[7] were performed. In patients with a transferrin isoelectrofocusing type 2 pattern, an O-glycosylation defect was investigated through the study of serum apolipoprotein CIII isoforms by isoelectrofocusing.[8]

In some patients with a type 1 pattern, phosphomannomutase (PMM2; Enzyme Commission number 5.4.2.8) and mannose-6-phosphate isomerase (Enzyme Commission number 5.3.1.8) activities were determined in fibroblasts and/or leukocytes, according to Van Schaftingen and Jaeken.[9] After excluding phosphomannomutase 2 and phosphomannose isomerase deficiencies, analysis of dolichol-linked oligosaccharides was carried out in fibroblasts of 5 patients with a type 1 pattern[10] and glycan structure analysis for 6 patients with a type 2 pattern.[11] Genetic testing was performed after written informed consent from the patients or their legal guardians. Pathogenic variants in *PMM2* were confirmed by Sanger sequencing, as previously reported.[12]

Most patients with non–PMM2-CDG were examined by a capture-targeted panel of 79 CDG-associated genes. The mean coverage in the target region was approximately ×600 and a genotype (homozygous reference, heterozygous or homozygous variant) was called for more than 95% of the targeted bases. Variant annotation and prioritization were done using the Cartagenia Bench tool. Analysis was done for the exonic sequences and 20 bp of the flanking intronic regions. In addition, deep intronic and promotor regions, in which variants were annotated in the Human Gene Mutation Database, were also scrutinized. An initial filtering of the variants was done based on the population frequency (<5% in at least 2 population databases). Non-informative cases were submitted to whole exome sequencing. Genomic DNA was sheared by sonication, platform-specific adaptors were ligated, and the resulting fragments were size selected. The library was captured using the SeqCap EZ Human Exome Library v 3.0 (Roche NimbleGen), and paired end (2 × 101 bp) sequenced on a HiSeq2000 (Illumina). Reads were aligned to the human reference genome (hg19) using Burrows-Wheeler Aligner (BWA v 0.6.2), and duplicate reads were removed using Picard MarkDuplicates (v 1.78). Local realignment around insertions and deletions, base quality score recalibration, and variant calling were performed using Genome Analysis Toolkit (v 2.4.9) RealignerTargetCreator, IndelRealigner, BaseRecalibrator, and UnifiedGenotyper. Variants were annotated using Annovar (v 11-02-2013). Variant prioritization

Table II. Allelic frequency of Portuguese patients wiht PMM2-CDG

Nucleotide changes	Protein changes	Allelic frequency (%)
c.422G>A	p.Arg141His	20.5
c.193G>T	p.Asp65Tyr	19.3
c.484C>T	p.Arg162Trp	14.1
c.470T>C	p.Phe157Ser	10.3
c.722G>C	p.Cys241Ser	7.7
c.368G>A	p.Arg123Gln	6.4
c.691G>A	p.Val231Met	5.1
c.367C>T	p.Arg123*	5.1
c.677C>G	p.Thr226Ser	2.6
c.338C>T	p.Pro113Leu	1.3
c.353C>G	p.Thr118Ser	1.3
c.395T>C	p.Ile132Thr	1.3
c.458T>C	p.Ile153Thr	1.3
c.550C>A	p.Pro184Thr	1.3
c.710C>T	p.Thr237Met	1.3
c.710C>G	p.Thr237Arg	1.3
c.523+3A>G		1.3

*Translation termination (stop) codon (https://www.hgvs.org/mutnomen/standards.html#aacode).

was applied, based on recessive inheritance, conservation, and pathogenicity prediction scores.

Results

Table I summarizes the main clinical and diagnostic data on the 63 patients, with 14 different disorders of protein N- and of protein N-combined with O-glycosylation (presented according to their subcellular location). Eleven of the 24 patients without PMM2-CDG and 15 of the 39 patients PMM2-CDG have been reported previously.[13-32]

Apart from 2 patients with SLC35A2-CDG (1 male and 1 female) diagnosed through a next-generation sequencing panel, the patients with CDG included in this work presented an increased %CDT and an abnormal transferrin isoform profile, even in adulthood.

Forty-three patients had a type 1 transferrin isoform profile, 14 patients had a type 2 profile and from the latter, 9

Table III. Updated version of the table presented in 2007 15 with Portuguese patients with PMM2-CDG (including HPO severity classification)*

Patients	Sex	Age at diagnosis	Nucleotide change	Protein	Clinical severity
P1	-	PND	c.422G>A; c.484C>T	p.(Arg141His); p.(Arg162Trp)	-
P2	F	1.5 y	c.422G>A; c.677C>G	p.(Arg141His); p.(Thr226Ser)	Moderate
P3	M	1 mo	c.470T>C; c.691G>A	p.(Phe157Ser); p.(Val231Met)	Profound
P4	-	PND	c.470T>C; c.691G>A	p.(Phe157Ser); p.(Val231Met)	-
P5	M	3 y	c.367C>T; c.484C>T	p.(Arg123*); (p.Arg162Trp)	Moderate
P6	M	3 mo	c.193G>T; c.368G>A	p.(Asp65Tyr); p.(Arg123Gln)	Profound
P7	-	PND	c.193G>T; c.368G>A	p.(Asp65Tyr); p.(Arg123Gln)	-
P8	F	2.5 y	c.422G>A; c.484C>T	p.(Arg141His); p.(Arg162Trp)	Moderate
P9	F	3 mo	c.422G>A; c.691G>A	p.(Arg141His); p.(Val231Met)	Profound
P10	M	1.5 y	c.422G>A; c.722G>C	p.(Arg141His); p.(Cys241Ser)	Moderate
P11	F	3 mo	c.193G>T; c.422G>A	p.(Asp65Tyr); p.(Arg141His)	Profound
P12	F	4 mo	c.193G>T; c.368G>A	p.(Asp65Tyr); p.(Arg123Gln)	Profound
P13	F	4 y	c.193G>T; c.193G>T	p.(Asp65Tyr); p.(Asp65Tyr)	Moderate
P14	F	2 y	c.470T>C; c.722G>C	p.(Phe157Ser); p.(Cys241Ser)	Moderate
P15	F	1 mo	c.193G>T; c.422G>A	p.(Asp65Tyr); p.(Arg141His)	Profound
P16	M	4 y	c.422G>A; c.484C>T	p.(Arg141His); p.(Arg162Trp)	Moderate
P17	F	16 y	c.338C>T; c.422G>A	p.(Pro113Leu); p.(Arg141His)	Moderate
P18	M	2 y	c.368G>A; c.458T>C	p.(Arg123Gln); p.(Ile153Thr)	Moderate
P19	M	3 mo	c.470T>C; c.691G>A	p.(Phe157Ser); p.(Val231Met)	Severe
P20	F	5 y	c.422G>A; c.484C>T	p.(Arg141His); p.(Arg162Trp)	Moderate
P21	M	18 y	c.470T>C; c.484C>T	p.(Phe157Ser); p.(Arg162Trp)	Mild
P22	F	1 y	c.422G>A; c.722G>C	p.(Arg141His); p.(Cys241Ser)	Moderate
P23	M	2 y	c.422G>A; c.677C>G	p.(Arg141His); p.(Thr226Ser)	Moderate
P24	M	1 mo	c.193G>T; c.422G>A	p.(Asp65Tyr); p.(Arg141His)	Profound
P25	M	1 mo	c.193G>T; c.367C>T	p.(Asp65Tyr); p.(Arg123*)	Profound
P26	-	PND	c.193G>T; c.367C>T	p.(Asp65Tyr); p.(Arg123*)	-
P27	M	11 y	c.193G>T; c.722G>C	p.(Asp65Tyr); p.(Cys241Ser)	Mild
P28	M	1 mo	c.193G>T; c.470T>C	p.(Asp65Tyr); p.(Phe157Ser)	Profound
P29	M	1 mo	c.193G>T; c.470T>C	p.(Asp65Tyr); p.(Phe157Ser)	Profound
P30	M	4 y	c.353C>G+c.550C>A; c.484C>T	p.(Thr118Ser)+p.(Pro184Thr); p.Arg162Trp	Mild/borderline
P31	F	3 y	c.367C>T; c.722G>C	p.(Arg123*); p.(Cys241Ser)	Mild
P32	F	3 y	c.193G>T; c.484C>T	p.(Asp65Tyr); p.(Arg162Trp)	Mild/borderline
P33	M	1 y	c.395T>C; c.523+3A>G	p.(Ile132Thr)	Moderate
P34	F	1 y	c.484C>T; c.710C>G	p.(Arg162Trp); p.(Thr237Arg)	Moderate
P35	F	8 mo	c.470T>C; c.710C>T	p.(Phe157Ser); p.(Thr237Met)	Severe
P36	M	19 y	c.422G>A; c.722G>C	p.(Arg141His); p.(Cys241Ser)	Moderate
P37	M	9 y	c.422G>A; c.484C>T	p.(Arg141His); p.(Arg162Trp)	Mild
P38	F	3 y	c.422G>A; c.484C>T	p.(Arg141His); p.(Arg162Trp)	Moderate
P39	M	1 mo	c.193G>T; c.368G>A	p.(Asp65Tyr); p.(Arg123Gln)	Profound

CNS, central nervous system; *HPO*, human phenotype ontology; *ID*, intellectual disability.
*Clinical severity classification is based on the degree of CNS involvement (ID) and involvement of other organs (heart, kidneys, liver).

also showed an abnormal apolipoprotein CIII isoform profile suggestive of an associated O-glycosylation defect. PMM activities in fibroblasts were measured in 18 patients with CDG-I, with activities ranging from undetectable to normal.[33] Serum dolichols were measured in 1 patient with ALG1-CDG revealing a very small increase of isoprenols. Sanger sequencing of *PMM2* confirmed the diagnosis of PMM2-CDG in 39 patients from 35 unrelated families. Seventeen pathogenic variants were detected: 15 resulting in an amino acid change (missense variants), 1 resulting in a stop codon (nonsense variant) and 1 causing a splice variant. The variants found in these patients with PMM2-CDG have been reported (**Table II**). All patients were compound heterozygotes except 1 that was homozygous for c.193G>T (p.D65Y), which is known to be a mild variant. These patients developed symptoms early in life, mostly hypotonia and failure to thrive (**Table III**). The phenotypic spectrum further comprised the known features with psychomotor disability, ataxia and intestinal, hepatic, cardiac, renal, and coagulation involvement.[34-37] Overall mortality rate was 18% (7 of 39) between the age of 1 month and 1 year. As for the patients without PMM2-CDG, a final diagnosis was obtained mostly by a combination of biochemical, molecular, or glycobiological investigations[26,27,30] supplemented in recent years by massive parallel sequencing either in selected panels (CDG, epilepsy, or clinical exome), or whole exome sequencing. The patients identified with this integrated approach included 1 ALG1-CDG, 1 ATP6AP1-CDG, 1 ATP2AP2-CDG, 2 ATP6V0A2-CDG, 1 CCDC115-CDG, 1 COG1-CDG, 1 COG4-CDG, 2 DPAGT1-CDG, 4 MAN1B1-CDG, 1 PGM1-CDG, 3 RFT1-CDG, 4 SLC35A2-CDG, and 2 SRD5A3-CDG.

Discussion

We compared the mutational spectrum of CDG found in Portuguese families with the one previously reported in a Spanish cohort.[38] Only 8 out of 18 CDG found in Spanish patients are shared by the Portuguese cohort (ALG1-CDG, ATP6V0A2-CDG, DPAGT1-CDG, PGM1-CDG, PMM2-CDG, RTF1-CDG, SLC35A2-CDG, and SRD5A3-CDG), which reveals a high level of genotypic differences between the 2 countries, despite their geographic proximity.

A more detailed analysis of the most frequent CDG, PMM2-CDG, revealed that there are 39 patients (from 35 families) with 17 different variants in the Portuguese cohort, compared with 71 patients with 37 variants in the Spanish cohort. The number of deleterious variants in distinct populations seems to reflect specific aspects of the population structure rather than any biological aspect related to *PMM2* itself; therefore, the genetic diversity associated with *PMM2* in each population is high.[3,34,39]

Portuguese and Spanish groups have 15 *PMM2* variants in common. The 2 most prevalent mutations are the c.422G>A (p.R141H) (20 %), the most common variant worldwide and the c.193G>T (p.D65Y) (18 %), an Iberian founder variant.[15] In a Scandinavian CDG cohort there are also 2 nearly equally prevalent mutations: the c.422G>A (p.R141H) and the c.357C>A (p.F199L), the latter being a South Scandinavian founder variant.[40] Note that, contrary to the c.422G>A (p.R141H)[41] variant, neither c.193G>T (p.D65Y) or c.357C>A (p.F199L) are lethal in the homozygous form.

As to the clinical presentations, there are no marked differences with the patients with CDG from other countries except for 5 patients. In our cohort of patients with PMM2-CDG, P30 and 32 (**Table III**) had an unusually mild/borderline phenotype. In addition, 2 RFT1-CDG adult patients with mild intellectual disability (from the same small region and thus, possibly related), do not present deafness or epilepsy, contrary to all other reported patients.[22] The fifth is a patient with cutis laxa because of ATP6V0A2-CDG but also corneal dystrophy necessitating repeated corneal transplantation.[24] This eye disorder has not been reported in other patients with this CDG.

Although the vast majority of the patients are still alive, 7 patients with PMM2-CDG and 1 with CCDC115-CDG died within their first year. One patient with ATP6V02-CDG died in his twenties. Our oldest patients with CDG are 37 and 39 years of age.

Because of their clinical and genetic heterogeneity, many CDG are difficult to recognize and to diagnose, whereas, vice versa, few types are sufficiently pathognomonic to drive specific genetic testing. Some phenotypes overlap with those of non-CDG disorders such as mitochondrial diseases.[42] There has been a significant increase in the number of detected CDG, particularly because the more generalized use of massive parallel sequencing. ■

We thank the patients and their families for their participation in this work, and the Portuguese Association for CDG and other Rare Metabolic Diseases. We also thank Dirk J. Lefeber and Luisa Sturiale for glycan structure analysis, Christian Thiel, and the late Christian Körner for lipid-linked oligosaccharide studies and Sofia Duarte and Diana Antunes for referring the patients. We thank Eugénia Pinto, Sónia Rocha and Domingos Sousa for the technical support.

Submitted for publication Aug 4, 2020; last revision received Nov 9, 2020; accepted Dec 10, 2020.

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