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ORIGINAL ARTICLE

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ALG8-CDG: Molecular and phenotypic expansion suggests clinical management guidelines

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Abstract

Congenital disorders of glycosylation are a continuously expanding group of monogenic disorders of glycoprotein and glycolipid glycan biosynthesis. These disorders mostly manifest with multisystem involvement. Individuals with ALG8-CDG commonly present with hypotonia, protein-losing enteropathy, and hepatic involvement. Here, we describe seven unreported individuals diagnosed with ALG8-CDG based on biochemical and molecular testing and we identify nine novel variants in *ALG8,* bringing the total to 26 individuals with ALG8-CDG in the medical literature. In addition to the typical multisystem involvement documented in ALG8-CDG, our cohort includes the two oldest patients reported and further expands the phenotype of ALG8-CDG to include stable intellectual disability, autism spectrum disorder and other neuropsychiatric symptoms. We further expand the clinical features in a variety of organ systems including ocular, musculoskeletal, dermatologic, endocrine, and cardiac abnormalities and suggest a comprehensive evaluation and monitoring strategy to improve clinical management.

Click here to access the podcast for this paper.

KEYWORDS
congenital disorders of glycosylation, lipid-linked oligosaccharides, N-glycans

1 | INTRODUCTION

Congenital disorders of glycosylation (CDG) are a rapidly expanding group of metabolic disorders characterized by multiorgan involvement with variable phenotypes. CDG are caused by impairment in glycosylation pathways, including N-glycosylation and O-glycosylation of proteins and lipids, as well as glycosylphosphatidylinositol (GPI) anchor synthesis. Among the different glycosylation defects, protein N-linked glycosylation defects are the most common and many N-glycosylation defects can be screened by analyzing the glycosylation status of the serum glycoprotein transferrin.

ALG8-CDG (OMIM #608104) is an autosomal recessive disorder caused by variants in *ALG8* on chromosome 11q14.1 encoding dolichyl-P-glucose: Glc-1-Man-9-GlcNAc-2-PP-dolichyl-alpha-3-glucosyltransferase (ALG8; 608103), an enzyme that attaches the second glucose residue to dolichol-PP-glycans in the endoplasmic reticulum (ER). Defects in *ALG8* lead to accumulation of $Glc_1Man_9GlcNAc_2$-PP-dolichyl (Figure 1). Nineteen individuals have been described with clinical spectra ranging from mild symptoms to death within the first hour of life. We describe an additional seven individuals with ALG8-CDG from six families with nine novel variants and expand its molecular, biochemical and clinical spectrum. We further provide recommendations to optimize the clinical management of ALG8-CDG.

2 | MATERIALS AND METHODS

2.1 | Inclusion criteria

A written consent to participate in the research study was obtained from the families under an IRB-approved by each institution or, when required, a Sanford Burnham Prebys IRB protocol. Inclusion criteria for this study

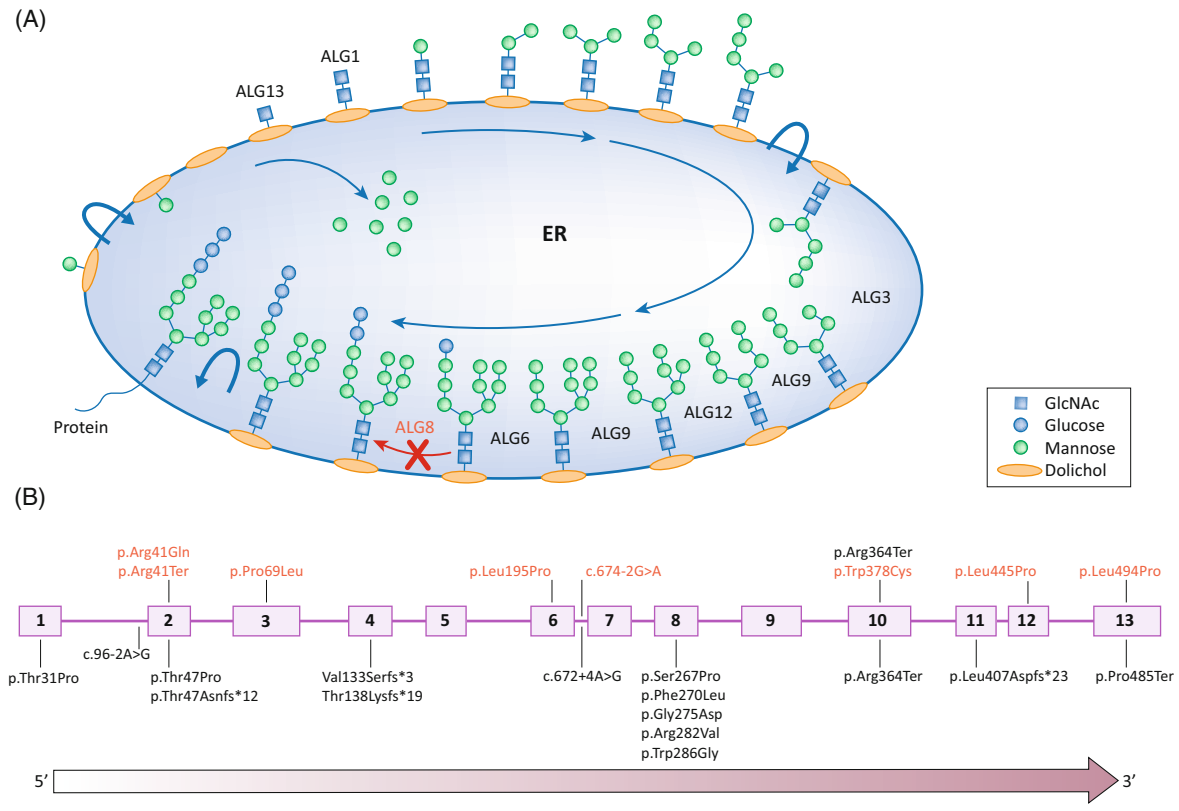

FIGURE 1 Biochemical and genetic disruption in ALG8-CDG.

TABLE 1 Overview of ALG8-CDG individuals

	P1	P2	P3	P4	P5	P6	P7
DNA variant[a]	c.122G > A/c.674 - 2G > A	c.122G > A/c.674 - 2G > A	c.121C > T/ c.122G > A	c.1090C > T/c.1134G > T	c.584 T > C/ c.1264 T > C	c.206C > T (homozygous)	c.1481 T > C/Del Ex3–13
Protein variation	p.Arg41Gln/splice acceptor p.LOF	p.Arg41Gln/splice acceptor p.LOF	p.Arg41Ter/ p.Arg41Gln	p.Arg364Ter/p.Trp378Cys	p.Leu195Pro/ p.Phe422Leu	p.Pro69Leu	p.Leu494Pro/ partial gene deletion of Ex3-13
Novel variant	Y/Y	Y/Y	Y/Y	N/Y	Y/Y	Y/Y	Y/Y
Age of diagnosis	6 years	3 years	5 years	7 years	3 years	9 years	3 years
Gender	M	M	M	F	M	M	M
Ethnicity	Caucasian	Caucasian	European	European, Turkish, South American	Korean	Italian	Moldavian
Family history	Affected brother (individual 2)	Affected brother (individual 1)	–	–	ND (adopted)	–	–
Dysmorphisms	+	+	+	+	+	+	+
Facial features	Prognathism	Down slanting palpebral fissures	Prominent brow, deep-set eyes, mild epicanthal folds	Macrocephaly, Coarse facial features, prognathism and hypertelorism	Hypoplastic midface, epicanthal fold, and hypertelorism	Prominent forehead, narrow, down slanting palpebral fissures, broad upturned nose, dysplastic ears, high-arched palate	Slightly prominent metopic ridge, partial epicanthal folds
Microcephaly	ND	ND	ND	–	–	–	–
Neurological abnormalities	+	+	+	+	+	+	+
Developmental delay/ intellectual disability	+/+ severe	+/+ severe	+/+ severe	+/+	+/+	+/+ severe	+/+
Seizure	+ (GTC, absence, myoclonic)	–	+ (febrile seizures, TC)	+ (febrile seizures)	+ (infantile spasms, myoclonic, TC, atonic)	+ (focal and bilateral TC)	± One suspected episode at 10 months

(Continues)

TABLE 1 (Continued)

	P1	P2	P3	P4	P5	P6	P7
Epilepsy treatment	Tegretol, lamictal	NA	Keppra	NA	Topamax, Keppra, clobazam, Vimpat, cannabinoid oil, ketogenic diet	Levetiracetam and clonazepam	−
Ataxia or gait problems	+	+	+	+	+	+	+
Hypotonia	+	+	+	+	+	+	−
Autism spectrum disorder	+	+	−	−	+ −	−	−
Behavioral concerns	+ Insomnia	+ Aggressive behaviors, Depression, ADHD, severe insomnia, hyperphagia	+ Developmental regression in setting of clostridium difficile infection	+ Autistic features	+ Deficits in social communication	+ Defiant oppositional disorder, Obsessive compulsive disorder, deficits in social communication (SCQ score 22 (normal ≤15)	+ Deficits in social communication (SCQ score 20 (normal ≤15)
Brain anomalies	−	−	−	NA	Thrombosis of the left transverse sinus left sigmoid sinus and left jugular bulb.	Cerebellar atrophy, cerebellar vermis hypoplasia, dilated Virchow-Robin spaces in the supratentorial white matter and basal ganglia	Slight expansion of the periencephalic frontal liquoral spaces bilaterally
Ocular impairment	+ (strabismus, Myopia)	−	−	+ (retinal hypopigmentation, blocked lacrimal duct)	+ (amblyopia, mild astigmatism, strabismus)	+ (myopia, strabismus)	−
Hepatopathy	+	+	+	+			+
Hypoalbuminemia	−	−	+	+ severe	+	−	−
Transaminitis	−	+	+	+ (intermittent elevations with illness)	+ (intermittent)	+	−

TABLE 1 (Continued)

	P1	P2	P3	P4	P5	P6	P7
Coagulopathy	+ high INR	−	+ (elevated aPTT, protein C deficiency, FIX deficiency, ATIII deficiency) easy bruising	+ prolonged aPTT and PT, decreased fibrinogen, FXI and ATIII	+ (low-protein S activity, low FX, low ATIII, high dAPC Resistance, high aPTT, high PT/INR, low-fibrinogen) venous thrombosis	+ (low AT III)	+ (low AT III, low protein C, low FXI, increased aPTT)
Other	−	−	−	−	+ (fatty liver on US)	+ (hepatomegaly)	−
Gastrointestinal	+	+	+	+	+	+	+
Faltering growth	−	−	−	+	+ (intermittent)	−	−
GI Problems/G-Tube	+ dysphagia	ND	−	+ Milk protein allergy/ NGT in infancy. Dysphagia	−	ND	+
Diarrhea /PLE	−	−	−	+	ND	+ (treated with lactose-free milk formula)	+ Resolved alternating episodes of constipation and diarrhea
chronic constipation	+	ND	+	+	+	−	−
GERD/vomiting	−	+/−	−	+/+	+/+ (intermittent)	ND/-	−
Cardiac Abnormalities	−	ND	+ (borderline normal diastolic function)	+ Bicuspid AV, narrowing of the aortic isthmus, RV hypertrophy, Prolonged QT	+ (trivial pericardial effusion; slight enlargement of aortic root, mild dilatation of his sinotubular junction and proximal ascending aorta and an S-shaped ventricular septum)	−	+ (long QT syndrome)
Respiratory	+ (apnea)	+ (asthma)	−	−	+ (RAD, breath holding spells, laryngomalacia)	+ (recurrent respiratory infections)	−
Muscular	+ Dysarthria, slow recovery from anesthesia	ND	+ Dysarthria, Muscle Weakness	+ CK elevation with illness, muscle Weakness, torticollis	+ Dysarthria, Muscle Weakness,	+ Dysarthria	−

(Continues)

TABLE 1 (Continued)

	P1	P2	P3	P4	P5	P6	P7
Skeletal	+	+	+	+	+	+	+
Scoliosis or Kyphosis	+ scoliosis	+ Kyphosis	+ scoliosis	−	+	+ Kyphosis	−
Joint abnormalities/ hyperextension of Joints	+ Elbow subluxations/+	+ disk herniation L5-S1	+ femoral head subluxation	+ partial congenital hip dislocation	−/+	−/+	−/+
Hand or foot abnormalities	ND	ND	ND	ND	−	+ Bilateral Pes Planus, bilateral shortened 4th and 5th metacarpal bones, brachydactyly	+ Bilateral Pes Planus
Fractures/ osteopenia	+ Recurrent fractures	+ Stress fracture T8-T9	ND	−	−	−	−
Hematological/ immunological	−	+	+ (anemia, thrombocytopenia)	+ Anemia; BT in infancy. Hypogammaglobinemia	+ (anemia, thrombocytopenia)	−	−
Recurrent infections/severe infection	−	+ Recurrent URI, tinea pedis, impetigo	ND	ND	+ (bronchitis, sinus infections, pertussis, acute otitis media)	+ (respiratory infection)	−
Genitourinary anomalies	+ Urinary incontinence	+ Urinary incontinence	−	+ Neurogenic bladder	−	+ Cryptorchidism	−
Renal abnormalities	+ Proteinuria	+ Hypernatremia, proteinuria	−	+ Renal cysts, nephrotic range proteinuria, hyponatremia	+ Proteinuria	−	−
Endocrine	Hypothyroidism, vitamin D deficiency hypertriglyceridemia	+ Hypercholesterolemia, hypertriglyceridemia, low LDL	−	+ hyperinsulinism/ hypoglycemia	+ Delayed bone age with low IGF-BP3, Hypercholesterolemia	−	−
NPCRS	ND	ND	17	18 at 12 years	22	17 at 15 years	8 at 6 years
Survival	Alive at 29.5 years	Alive at 27 years	Alive at 14 years	Alive at 12 years	Alive at 10 years	Alive at 15 years	Alive at 6 years

Abbreviations: ADHD, attention deficit hyperactive disorder; APTT, activated partial thromboplastin time; ATIII, antithrombin III; AV, aortic valve; BT, Blood transfusion; CK, creatine kinase; F, female; FIX, FX, FXI, Factor IX, X, XI; GTC, generalized tonic clonic; L5, lumber vertebra5; M, male; mo, months; N, no; NA, not applicable/not available; ND, not determined; NGT, nasogastric tube; NPCRS, Nijmegan pediatric CDG rating scale; P, Patient, PT, prothrombin time; RAD, Reactive Airway Disease; RV, right ventricle; S1, Sacrum vetebra1; T8,T9, thoracic vertebra8,9; TC, tonic–clonic; URI, upper respiratory infection; Y, yes;.

[a]The reference transcript is NM_024079.5.

required: (1) At least one abnormal biochemical test result indicating a CDG-I and (2) a molecular test result identifying homozygous or compound heterozygous variants in *ALG8*.

2.2 | Clinical studies

Clinical laboratory analysis and imaging was obtained as indicated. To evaluate the glycosylation status of secreted glycoproteins, we studied the N-glycan profile of two individuals (P3 and P5) using a clinically validated quantitative N-glycan assay.[1] Seven individuals underwent carbohydrate deficient transferrin (CDT) testing using a mass spectrometry approach (LC-ESI-TOF/MS)[2,3] or transferrin isoelectric focusing. All the individuals had the diagnosis confirmed by next generation sequencing using either gene panel (P3, P6, and P7), exome sequencing (P1, P2, P4, and P5), or CGH-array testing (P7). LLO analysis was performed by metabolic labeling with ^{3}H-mannose.[4]

3 | RESULTS

3.1 | Molecular analysis

Nine novel variants were seen in our cohort as shown in Table 1 and illustrated in Figure 1B. Varsome browser was used to predict variant pathogenicity[5] using ACMG guidelines for interpretation of sequence variants[6] along with transferrin glycosylation studies showing a type 1 pattern. The ALG8 (UNIPROT: Q9BVK2) variants identified in our cohort are localized either within predicted transmembrane domains (TMD4-p.Leu195Pro; TMD7-p.Trp378Cys; TMD9-p.Leu445Pro; TMD11-p.Leu494Pro) or within the cytoplasmic loops between TMDs (p.Arg41Gln, p.Arg41Ter, p.Arg364Ter, and p.Pro69Leu). Further, a splice acceptor variant (c.674-2G > A) was identified in P1 and P2, and a partial gene deletion of the *ALG8* Ex3-13 was identified in P7. In addition to *ALG8* pathogenic variants, P5 is heterozygous for *LDLR* (606945): c.178C > T (p. Gln60ter) associated with familial hypercholesterolemia.

3.2 | Biochemical studies

Fibroblasts from five patients (P1, P2, P3, P4, and P5) with suspected ALG8-CDG were analyzed for synthesis of LLO by metabolic labeling with ^{3}H-mannose. Each subject demonstrated accumulations of Man9GlcNAc2 and Glc1Man9GlcNAc2, in contrast to the predominant glucosylated Glc3Man9GlcNAc2 LLO observed in control cells, consistent with impairment of ALG8 activity.

All of the individuals in the cohort showed a type 1 serum transferrin pattern. Analysis of total plasma N-glycans using our recently described and clinically validated quantitative N-glycan assay in two subjects (P3, P5) did not show consistent or diagnostic deviations from control subjects (Table S1).

3.3 | Clinical features

We identified seven individuals (six male and one female) from six families with varying ethnic backgrounds. The ages in our cohort range from 6 to 29 years with all seven individuals currently alive. Members of our cohort had delayed diagnosis made between the ages of 3 and 9 years. Patients P4 and P6 had abnormal biochemical testing at age of 4 months and 23 months but were not molecularly diagnosed until ages 7 and 9 years, respectively. P6 was initially diagnosed with CDG-Ix, before molecular testing confirmed the diagnosis of ALG8-CDG. Summary of the clinical features of the seven new ALG8-CDG individuals is presented in Table 1 with further phenotypic information about adult patients included in supplemental material.

Individuals with ALG8-CDG have been reported to commonly present with hypotonia, protein-losing enteropathy (PLE), and hepatic involvement. In our cohort, hypotonia was noted in 6/7 with all exhibiting delayed psychomotor development. All seven individuals learned to walk, albeit with ataxia or otherwise abnormal gait. Previously unreported neurodevelopmental disorders, including autism spectrum disorder, were present in our cohort (5/7). Diagnosis of autism spectrum disorder was made following formal developmental assessment by a specialist (P1 and P2). P6 and P7 were assessed by social communication questionnaire with scores of 22 and 20 respectively (cutoff> = 15), but they did not receive a formal diagnosis of autism spectrum disorder due to the presence of autism symptoms in the context of syndromic intellectual disability. Additional behavioral concerns included aggressive behavior, oppositional defiant disorder, obsessive-compulsive disorder, attention deficit hyperactivity disorder, insomnia, and hyperphagia. In contrast to prior reports, PLE was not documented in our cohort. Hepatic involvement consisted of hypertransaminasemia (5/7), abnormal coagulation profile (6/7), and hypoalbuminemia (3/7).

Additional clinical features in our cohort, which have not been previously highlighted, include anomalies of the skeletal system (7/7) with our subjects exhibiting short stature, scoliosis or kyphosis, hyperextension and subluxation of joints, herniated disk, hip dysplasia, or recurrent fractures. We also observed previously unreported muscular problems including muscle weakness (4/7) and

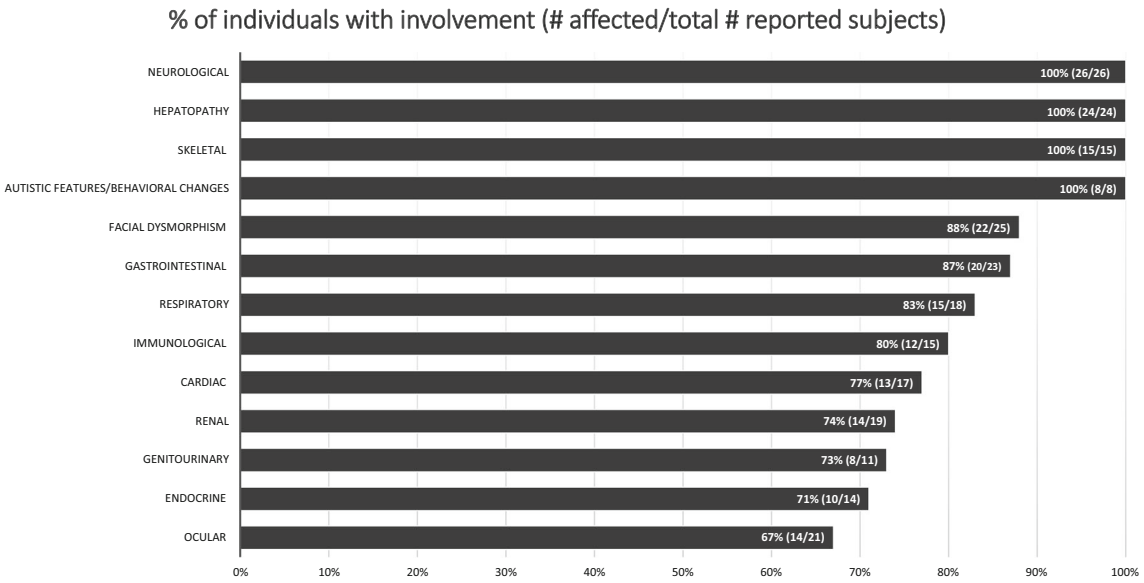

FIGURE 2 Clinical summary of all reported ALG8-CDG individuals.

elevated creatine kinase during illnesses in one individual (P4). Endocrinologic issues not previously reported included low IGF-BP3 (1/7) and transient hyperinsulinemia with episodes of hypoglycemia (1/7).

3.4 | Nijmegen scores

Using the Nijmegen Pediatric CDG Rating Scale (NPCRS),[7] five individuals were evaluated at their last clinical assessment. Most of the individuals (P3, P4, P5, and P6) scored in the moderate range (between 18 and 22) while P7 scored in the mild range (Table 1). NPCRS scores were not available for P1 and P2.

4 | DISCUSSION

Our description of seven new individuals with ALG8-CDG brings the total reported patients to 26.[8–20] Our cohort includes the two oldest patients reported (29.5 and 27 years). We reviewed the molecular, biochemical, and clinical findings in the 19 previously reported individuals with ALG8-CDG (Table S2).

Most *ALG8* pathogenic variants are family specific. Previously, 15 different *ALG8* pathogenic variants were identified. Nine novel variants were observed in our cohort, bringing the total to 24, including a majority present in compound heterozygosity (only four variants have been found in homozygosity: p.Thr47Pro, p.Trp286Gly, p.Ala282Val, and p.Pro69Leu). These variants were mostly missense (75%) and scattered throughout the gene. The most frequent variant, p.Thr47Pro, is present in 7/25 patients. Of the 26 patients, 17 were male (65%), and there were six sibling pairs. We could not identify a genotype–phenotype correlation among patients, demonstrating variability in the clinical features, even within families with identical genotypes.

The frequency of phenotypic features in all patients with ALG8-CDG is shown in Figure 2. All ALG8-CDG patients displayed abnormal neurological features (26/26). Our cohort expands the phenotype of ALG8-CDG to include stable intellectual disability, autism spectrum disorder and other behavioral concerns. Epilepsy was reported in six patients with ALG8-CDG[8,10,16,18,19] and was seen in five patients in our cohort without consistent seizure semiology. There does not appear to be a clear pattern of response to a particular antiepileptic drug (AED). In our cohort, the combination of carbamazepine and lamotrigine for (P1), levetiracetam and clonazepam for (P6), and levetiracetam for (P3) were required to improve seizure control. In P5, multiple AEDs were tried, but only ketogenic diet showed significant improvement in the seizure control.

The second most reported clinical manifestation was hepatic involvement (24/24). Severe hepatic and gastrointestinal involvement include life-threatening edema, ascites, and coagulopathy, leading to significant morbidity and mortality in 75% of the patients. In our cohort, the hepatic and gastrointestinal symptoms were less severe, three patients had hypoalbuminemia, but none of them developed ascites or edema. In our cohort, one of the six patients with coagulopathy developed venous thrombosis of the left transverse and sigmoid sinus and left jugular bulb. In previous reports, coagulopathy was complicated

TABLE 2 Suggested guidelines for evaluation and follow-up of patients diagnosed with ALG8-CDG

Evaluation		Baseline evaluation at diagnosis	First year of life		From 1 year to 5 years of age			After 5 years of age
			Every 3 months	Every 6 months	Every 3 months	Every 6 months	Yearly	
CDG testing	CDT	X		X			X	Yearly[a]
	N-glycan	X		X			X	Yearly[a]
Liver evaluation	AST/ALT	X	X[a]			X[a]		Yearly[a]
	PT/INR	X	X[a]			X[a]		Yearly[a]
	Albumin	X	X[a]			X[a]		Yearly[a]
	US and elastography	X		X			X	Yearly[a]
Hematological evaluation	CBC	X		X[a]		X[a]		Yearly[a]
	PT/PTT	X		X[a]		X[a]		Yearly[a]
	ATIII	X		X[a]		X[a]		Yearly[a]
	FXI	X		X[a]		X[a]		Yearly[a]
Renal evaluation	Urinalysis	X		X[a]		X[a]		Yearly[a]
	Renal US	X[a]						
Endocrine evaluation	TFT	X	X[a]				X[a]	Yearly[a]
	Adrenal axis	X	X[a]				X[a]	Yearly[a]
	Growth factors	X	X[a]				X[a]	Yearly[a]
	Lipid profile	X						Yearly[a]
	Bone density	X						Every 5 years[a]
Immunological evaluation	Hx of recurrent infections	X	X			X		Yearly
	IgA,E,G,M	X[a]						
	Response to vaccines	X						
Cardiology evaluation	Echo	X[a]					X[a]	Every 5 years[a]
	ECG							
Neurological evaluation	Brain MRI	X[a]						
	EEG	X[a]						
Ophthalmological evaluation		X					X[a]	Yearly[a]
Physical exam for skeletal anomalies		X						Yearly[a]
Nutrition and growth assessment		X	X[a]		X[a]			Yearly[a]
Development assessment	PHT/OT/ST	X	X		X			Yearly[a]

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; ATIII, antithrombin III; CBC, complete blood count; CDT, carbohydrate deficient transferrin; ECG, electrocardiogram; Echo, echocardiogram; EEG, electroencephalogram; FXI, factor XI; Hx, history; Igs, immunoglobulins; INR, international normalized ratio; MRI, magnetic resonance imaging; OT, occupational therapy; PHT, physical therapy; PT, prothrombin time; PTT, partial thromboplastin time; ST, speech therapy; US, ultrasound; WBC, white blood count.

[a]Minimum interval for evaluation, but more frequent if clinically indicated after this.

with vitreous bleeding during cataract operation, thrombosis of the inferior vena cava, hematemesis, and bloody diarrhea in four patients. Given the previously reported dissociation between liver ultrasound and transient elastography,[21] ALG8-CDG individuals should have regular liver ultrasound and liver elastography evaluations.

Additionally, gastrointestinal problems are frequent in ALG8-CDG (20/26) and include poor growth (10/18), feeding difficulties (7/14), PLE (6/13), chronic constipation (5/7), and gastroesophageal reflux disease (3/6).

Skeletal abnormalities are frequent in ALG8-CDG, emphasizing the importance of glycosylation in the development of cartilage and bone and in skeletal patterning pathways. Hypoglycosylation leads to defective remodeling resulting in osteopenia and fractures in patients with CDG.[22] In our two eldest patients (P1, P2), recurrent fractures and thoracic vertebral stress fracture were noted respectively. Rickets and generalized osteopenia were previously reported in one patient each, with low calcium and magnesium and reduced tubular reabsorption of phosphate identified in the patient with rickets.[20] Metabolic evaluations in our patients identified low vitamin (25-OH)D in P1, low calcium with normal alkaline phosphatase in P4, and elevated urine calcium in P5.

Facial dysmorphism identified in (22/25) may be a diagnostic clue for ALG8-CDG but does not demonstrate recognizable features. The most common features include epicanthal folds and hypertelorism in (6/25) with a wide variation of other dysmorphic features. In contrast to the previous reports of individuals with microcephaly, our cohort identified one patient with macrocephaly, and none with microcephaly. Skin findings were frequently observed with pale skin and excessive skin wrinkling the most common features. Inverted nipples were reported in four patients and abnormal fat distribution in eight patients was noted above and lateral to the buttocks, arms, axillae, or chest.

The immune pathophysiology of patients with CDG is explained by the essential and widespread role of glycosylation in the immune response. Abnormal glycosylation can affect cell–cell interactions, pathogen recognition, and cell activation leading to immunological dysfunction.[23] Among the 19 patients previously reported with ALG8-CDG, two presented with severe infections,[16] leading to death in one patient,[11] and one had increased inflammatory markers.[9] In our cohort, 3/7 patients presented with recurrent infections. In three patients, infection was identified as a trigger of clinical features such as developmental regression, worsening hypotonia, and seizures.

Cardiac abnormalities in our cohort further expand the phenotype and bring the total patients with reported cardiac involvement to 77% (13/17). Structural abnormalities include ventricular septal defect and patent ductus arteriosus in two patients and cardiomyopathy in one patient. In our cohort, other structural abnormalities include bicuspid aortic valve and narrowing of the aortic isthmus along with ventricular hypertrophy, enlargement of aortic root, and proximal ascending aorta and an S-shaped ventricular septum. Abnormal heart rhythm, including prolonged QT in three patients, bradycardia, and ventricular conductive block were noted in two patients with ALG8-CDG. Among these patients, none reported requiring a pacemaker.

Fourteen patients with ALG8-CDG had renal involvement, including proteinuria in seven patients, progressing to nephrotic syndrome in two patients, and tubulopathy with electrolyte disturbance. Renal cysts were identified in one patient in our cohort, while three previous patients reported microcystic kidney,[13] cortical microcyst,[18] and renal tubular dysgenesis.[16] It has been proposed that reduced heparan sulfate in the glomerular basement membrane and enterocyte may contribute to the proteinuria and PLE,[24,25] which may explain this feature in ALG8-CDG.

Glycosylation is important for the stability and function of endocrinologic factors. One of our patients had transient hyperinsulinism and recurrent episodes of hypoglycemia at 3 months and again at 6 years despite appropriate weight gain. Hypoglycemia was also reported in one previous patient. Although hyperinsulinism is a known etiology of hypoglycemia in some CDG, the pathophysiology in ALG8-CDG has not been defined clearly. Further, low IGF-BP3 was seen in one patient consistent with the in vivo study where the impaired glycosylation leads to reduction in the levels of IGF system including IGF-BP3.[26] Thyroid function tests are frequently abnormal in children with CDG because of abnormal glycosylation of TSH and thyroid-binding globulin. Hypothyroidism was identified in 7/14 patients which highlights hypothyroidism as a recurring feature of ALG8-CDG.

We also note lipid abnormalities in our cohort. Hypocholesterolemia is frequently described in patients with CDG-I. The hypercholesterolemia in P5 can be attributed to the LDLR mutation. However, the adult patients also demonstrate hypertriglyceridemia with P2 being overweight. It is unclear if patients with ALG8-CDG are more likely to acquire dyslipidemia and this points out the need to more fully understand the relationships between specific glycoproteins and their effects on lipoprotein metabolism across the lifespan[27] and to assess for dyslipidemia in patients with ALG8-CDG.

Ocular involvement is common and noted in 67% (14/21) of ALG8-CDG patients. Cataract, which has been described previously in six patients, was not present in our cohort. Retinitis pigmentosa was identified in two previous patients at the age of 8 years and at 4 months based on low vision and slight anomalies in the electroretinography respectively.[10,15] Other types of ocular pathology, such as microphthalmy, optic nerve atrophy, and abnormal eye movement, have been reported as well.

In summary, in light of commonly reported multisystem involvement in ALG8-CDG which may first manifest or worsen at any age, and given their significant impact,

we recommend a thorough evaluation for newly diagnosed patients to identify involved organ systems and establish appropriate care with specialist providers. Clinical evaluation and monitoring should include developmental assessment with the initiation of physical, occupational, and speech therapies and attention to behavioral issues in older children with referral for intervention (if needed), careful history to detect the presence of seizures and perform EEG, liver US and elastrography, evaluation by a licensed dietitian to monitor nutritional and growth status, cardiac examination with ECG and echocardiogram, detailed ophthalmologic exam, renal US, scoliosis screening, serial bone density measurements, careful history to evaluate for immunological involvement in the setting of recurrent infections, and serial laboratory assessments (see Table 2).

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CONFLICT OF INTEREST

Daniah Albokhari, Bobby G. Ng, Earnest James Paul Daniel, Lynne Wolfe, Kimiyo M. Raymond, Miao He, Nicole M. Engelhardt, Rita Barone, Agata Fiumara, Livia Garavelli, Gabriele Trimarchi, Eva Morava, Christina Lam, and Andrew C. Edmondson declare that they have no conflicts of interest. Hudson H. Freeze is a consultant for Avalo Therapeutics.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

ETHICS STATEMENT

This study was performed in accordance with ethical principles for medical research outlined in the Declaration of Helsinki. This study was approved by the institutional review board of the respective institutions.

INFORMED CONSENT

Written informed consent was obtained from all patients' guardians before inclusion in the study.

ANIMAL RIGHTS

This article does not contain any studies with animal subjects.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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