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Expanding the phenotype, genotype and biochemical knowledge of ALG3-CDG

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AUTHOR CONTRIBUTIONS

Hind Alsharhan provided clinical evaluations, drafted the initial manuscript, and revised the manuscript. Bobby G. Ng, Earnest James Paul Daniel, Jennifer Friedman, Eniko K. Pivnick, Amal Al-Hashem, Eissa Ali Faqeih, Nicole M. Engelhardt, Kierstin N. Keller, Jie Chen, Pamela A. Mazzeo, Michael J. Bamshad, Deborah A. Nickerson, Kimiyo M. Raymond, Hudson H. Freeze, Andrew C. Edmondson, and Christina Lam provided clinical evaluations, critically reviewed, and revised the manuscript. Pengfei Liu and Jill A. Rosenfeld performed NGS analysis and revised manuscript. Bobby G. Ng performed NGS and LLO analysis, Miao He provided N-glycan analysis and interpretation, critically reviewed, and revised the manuscript. University of Washington Center for Mendelian Genomics (UW-CMG) performed exome sequencing in multiple cases.

CONFLICT OF INTEREST

Dr. Friedman holds shares in Illumina and her Spouse is Founder and Principal of Friedman Bioventure, which holds a variety of publicly traded and private biotechnology interests. Hind Alsharhan, Bobby G. Ng, Earnest James Paul Daniel, Jennifer Friedman, Eniko K. Pevnick, Amal Al-Hashem, Eissa Ali Faqeih, Nicole M. Engelhart, Kierstin N. Keller, Jie Chen, Pamela A. Mazzeo, Jill A. Rosenfeld, Michael J. Bamshad, Deborah A. Nickerson, Kimiyo M. Raymond, Miao He, Andrew C. Edmondson, and Christina Lam declare that they have no conflicts of interest. Hudson H. Freeze is a consultant for Cerecor, Inc.

ETHICS APPROVAL AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.[20] Informed consent was obtained from all patients for being included in the study. Proof that informed consent was obtained must be available upon request if doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach, and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study. Additional informed consent was obtained from all patients for which identifying information is included in this article.

ANIMAL RIGHTS

This article does not contain any studies with animal subjects.

SUPPORTING INFORMATION

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

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Abstract

Congenital disorders of glycosylation (CDGs) are a continuously expanding group of monogenic disorders of glycoprotein and glycolipid biosynthesis that cause multisystem diseases. Individuals with ALG3-CDG frequently exhibit severe neurological involvement (epilepsy, microcephaly, and hypotonia), ocular anomalies, dysmorphic features, skeletal anomalies, and feeding difficulties. We present 10 unreported individuals diagnosed with ALG3-CDG based on molecular and biochemical testing with 11 novel variants in *ALG3*, bringing the total to 40 reported individuals. In addition to the typical multisystem disease seen in ALG3-CDG, we expand the symptomatology of ALG3-CDG to now include endocrine abnormalities, neural tube defects, mild aortic root dilatation, immunodeficiency, and renal anomalies. N-glycan analyses of these individuals showed combined deficiencies of hybrid glycans and glycan extension beyond $Man_5GlcNAc_2$ consistent with their truncated lipid-linked precursor oligosaccharides. This spectrum of N-glycan changes is unique to ALG3-CDG. These expanded features of ALG3-CDG facilitate diagnosis and suggest that optimal management should include baseline endocrine, renal, cardiac, and immunological evaluation at the time of diagnosis and with ongoing monitoring.

Keywords

congenital disorders of glycosylation; endocrine; immunodeficiency; neural tube defect; N-glycans

1 | INTRODUCTION

Congenital disorders of glycosylation (CDGs) are a rapidly expanding group of metabolic disorders with defects in the synthesis of glycans and their attachment to proteins and lipids. The first CDG was reported in 1980,[1] and now more than 140 types are known. This expansion in diagnosis has been largely due to the advances in both glycosylation biochemical testing and exome sequencing (ES). CDG frequently cause significant neurologic dysfunction with variable impairment of other organ functions.

ALG3-CDG (OMIM #60110) is one of the rare types of CDG, previously called CDGS-IV or CDG-Id, and was first reported in 1995[2, 3] as an autosomal recessive disorder. *ALG3* encodes for an alpha-1,3-mannosyltransferase, the first dolichol phosphate mannose (Dol-P-Man)-dependent mannosyltransferase involved in N-glycan synthesis. It transfers a mannose from Dol-P-Man onto the lipid-linked oligosaccharide (LLO) intermediate, $Man_5GlcNAc_2$-PP-dolichol in the lumen of the endoplasmic reticulum. Defects in ALG3 result in an accumulation of $Man_5GlcNAc_2$-PP-dolichol (Figure 1). Similar to other CDG, the reported clinical spectrum of ALG3-CDG includes multisystem involvement. The typical clinical manifestations of ALG3-CDG include severe neurological involvement, craniofacial abnormalities, facial dysmorphism, skeletal anomalies, ophthalmologic impairment, and feeding problems (Figure 2; Supplementary Table 2). The majority of reported pathogenic variants are missense, followed by small numbers of nonsense and splice junction variants. There is currently no treatment or cure for ALG3-CDG, and the management is largely supportive in nature, anticipating and addressing the various multisystem manifestations of the disorder. Here, we report 10 new individuals with ALG3-CDG with 11 novel variants and additional clinical findings to further expand the phenotype. Furthermore, we recommend monitoring evaluations to optimize clinical management.

2 | MATERIALS AND METHODS

2.1 | Individuals

Families provided written consent for participating in the study and obtaining clinical photography under an IRB approved in accordance with each individual's primary physician or, when required, a Sanford Burnham Prebys Medical Discovery Institute IRB protocol. Inclusion criteria required molecular findings showing the presence of homozygous or compound heterozygous variants in *ALG3* with biochemical findings indicating type I CDG when possible.

2.2 | Clinical studies

All patients were clinically evaluated by clinical geneticists at their respective tertiary healthcare centers. Patients underwent comprehensive history and physical examinations, fasting blood chemistry, and genetic testing. In some patients, X-ray, echocardiography, abdominal ultrasonography, electroencephalogram (EEG), and brain MRI scans were also performed when determined to be clinically necessary.

2.3 | Biochemical studies

We quantitatively analyzed the N-glycan profile of total plasma glycoproteins from four of the individuals (P1, 2, 3, 4) using a clinically validated N-glycan assay.[19] CDT testing was performed in eight individuals (P1, 2, 3, 4, 6, 7, 8, 9) by mass spectrometry (LC-ESI-TOF/MS).[20,21] Nine individuals underwent clinical genetic testing by next generation sequencing, using either gene panels for intellectual disability (P1, 3), or ES (P2, 4, 5, 7, 8, 9, 10). P6 was diagnosed through Sanger sequencing. *VANGL2* gene sequencing was performed for P3 using PCR amplified and capillary sequencing and analyzed for sequence variants. LLO studies for P1, P8, and P9 were done after molecular diagnosis, while P6 was analyzed prior to identifying *ALG3* variants. LLO analysis was performed by metabolic labeling with ^{3}H-mannose.[22]

3 | RESULTS

3.1 | Molecular findings

We identified 11 novel variants in our cohort as shown in Table 1 and illustrated in Figure 1. We predicted the pathogenicity of these novel variants using Varsome tool[23] that is based on the ACMG Standards and guidelines for interpretation of sequence variants[24] together with glycosylation studies confirming type I CDG (Table 1). The vast majority of *ALG3* (UNIPROT - Q92685) variants identified in the study lie within predicted transmembrane domains (TMD1 - p.W24X; TMD3 - p.Y132C, p.L137_L138INSVFLL; TMD5 - p.N174S; TMD6 - p.L219P; TMD7 - p.A204V, p.L250Q, p.L251P; and TMD8 - p.A305D). The p.R171Q, p.R266C, p.Q331X, and p. R385T all occur within predicted cytoplasmic loops between TMDs.

Individual 2, 3, and 5 are homozygous for previously described pathogenic variants (Table 1). In addition to *ALG3* variants, P2 is heterozygous for a paternally inherited variant of uncertain significance in *VANGL2*, c.1211 A>G [p.Lys404Arg] in exon 7 (NM_020335.2) that could be associated with increased risk for nonsyndromic neural tube defects (NTDs) with incomplete penetrance.[25,26] This variant was not detected in his distantly related P3. P10 has inherited homozygous variants, while his unaffected sibling is heterozygous for this variant.

3.2 | Clinical findings

Summary of the clinical manifestations of the 10 new ALG3-CDG individuals is presented in Table 1 and Figure 2. In total, we studied 10 individuals (4 male/6 female) ranging from newborn to 37 years old, from nine families with different ethnic backgrounds. P8 and P9 are siblings with novel inherited variants. Seven of the individuals are alive with the exception of P5, 6, and 10. P5 was stillborn and carried a previously reported homozygous pathogenic variant (p.R171Q).[4,11] P6 passed away at the age of 1-year secondary to multiorgan failure. P10 died soon after birth due to the complication of multiple congenital anomalies (MCA) and hydrops. While pregnancies were variably complicated by polyhydramnios, intrauterine growth restriction, hydrops, fetal akinesia, pyelectasis, myelomeningocele (MM), maternal gallstone pancreatitis, hyperemesis, and gestational diabetes, there was no consistent pattern in prenatal complications among the 10 individuals.

The degree of intellectual disability varied significantly within the cohort, with some individuals exhibiting profound developmental delays in all domains, while others achieved, as adults, neurocognitive abilities equivalent to an average age of 7 to 9 years. An illustration of the mild end of the neurocognitive spectrum is publicly available in a televised interview with P8 by “The Atlantic Live” (https://www.youtube.com/watch?v=Zqu_aC9TrhY).

Strabismus and optic atrophy were the most frequent ocular abnormalities. Specific facial dysmorphism were not consistent. The most frequent features included epicanthal folds, downslanting palpebral fissures, broad/flat nasal bridge, high palate, micrognathia, and dysplastic ears (Supplementary Figure 1). Skeletal abnormalities were observed in eight of our cohort (Supplementary Figure 1) in form of arthrogryposis, scoliosis, club feet, hip dysplasia, camptodactyly, contractures, overlapping digits, and talipes. Of those with contractures and were still living, all received physical and occupational therapy, and none have yet undergone surgical treatment.

Gastrointestinal problems were observed in (8/10) including feeding difficulties (6/8) and failure to thrive (FTT) (4/8) (weight < second percentile) requiring tube feeding (3/8). Hepatopathy consisted of mild trans-aminitis (ALT 47-149; reference range [RR] 6-40 IU/L, AST 55-128; RR 5-41IU/L) and hypoalbuminemia (2.6-3.7; RR 3.8-5.4 g/dL). Low antithrombin III (43-59; RR: 86%-145%), decreased factor XI (44%-46%; RR 63%-142%), and prolonged APTT (37.2-58; RR 22-36 seconds) were the most common hematologic defects. Previously unreported endocrine abnormalities were present in more than half of our cohort, including central hypothyroidism, central adrenal insufficiency, growth hormone deficiency, and subsequent hypoglycemia. Hypolipidemia was observed in three subjects.

Both upper and lower respiratory infections were the predominant types of recurrent infection, mainly in P2 and P3. Both P2 and P3 had immunologic evaluation demonstrating normal IgG, IgA, and IgM levels with protective Tetanus and Hib titers following immunization. Absolute T, B, and Natural Killer cell numbers were normal. P2 had normal neutrophil oxidative burst. However, P2 was diagnosed with specific antibody deficiency due to rapid loss of vaccination-induced protection against pneumococcus, decreasing from protective immunoglobulin titers against 13/14 serotypes to protective titers against only 5/14 serotypes within a few months of vaccination). Both P2 and P3 had an advanced for age naive to memory T cell ratio at 1.7:1 (P2) and 5:1 (P3). P2 was diagnosed with specific antibody deficiency and is maintained on weekly subcutaneous IG therapy and Azithromycin prophylaxis. On the other hand, P3 (who has the same homozygous variant) had recurrent infections frequently requiring hospitalization, but a normal immunological work up. She had a recent hospitalization with SARS-CoV-2 infection with fever but without respiratory exacerbation, in contrast to a preceding hospitalization due to Adenovirus and Coronavirus HKU1 coinfection resulting in intensive care unit admission with intubation due to respiratory failure, which was complicated by sepsis, disseminated intravascular coagulation and tracheitis.

Half of our subjects (5/10) had a delayed diagnosis made between the ages of 4 to 33 years primarily by molecular testing followed by abnormal biochemical testing confirming type I glycosylation defects. This delay was attributed mainly to lack of common features of CDG

(P4), accessibility to the molecular testing as well as the lack of such testing in the early 1990s.

3.3 | Nijmegen scores

Eight individuals were evaluated using the Nijmegen Pediatric CDG Rating Scale at their last clinical assessment (NPCRS),[27] which scales subjects into mild (0-14), moderate (15-25), or severe (26-76/82 [0-2 years/>2 years]) categories. Most of the individuals scored in the severe range with an average score of 31 (Table 1). Scores were not available for P5 and P10 as one was stillborn and the other died soon after birth.

3.4 | Synthesis of abnormal lipid linked oligosaccharide (LLO) N-glycosylation precursor

Fibroblasts from four of the suspected ALG3-CDG cases were analyzed for synthesis of LLO by metabolic labeling with ^{3}H-mannose. ALG3 is required for synthesis of full-size LLO. Each subject accumulated a $Man_5GlcNAc_2$ size glycan, consistent with the size predicted for pathological mutations in *ALG3*.

3.5 | Abnormal serum transferrin

CDT testing was performed for eight individuals. Type I pattern was consistently seen, similar to previously reported individuals with ALG3-CDG, with increased mono:di-glycosylated (M/D) ratio, indicating the absence of entire N-glycans on a portion of the molecules. Of note, we found a positive correlation between M/D ratio and NPCRS score but it did not reach statistical significance (data not shown). Similarly, M/D ratio in P2 and P3 had improved over time (Table 1).

3.6 | Abnormal plasma N-glycan profiles in ALG3-CDG

In addition to the partial absence of occupied N-glycosylation sites in transferrin, the synthesis of truncated LLO precursors in ALG3-CDG would predict that processing and/or extension of those N-glycans would be altered and provide additional diagnostic biomarkers. Analysis of total plasma N-glycans showed significant increases of N-linked small high mannose species (including $Man_{0\text{-}4}GlcNAc_2$) in all four individuals (1-4, and) who were available for testing, along with reduced $Man_9GlcNAc_2$ (Supplementary Table 1). Two hybrid glycans that whose increases are diagnostic for Man1B1-CDG,[28] were both deficient in all four subjects. Interestingly, $Man_5GlcNAc_2$ abundance was not increased in the plasma from these individuals. $Man_6GlcNAc_2$ was reduced in 2/4 subjects while $Man_7GlcNAc_2$ was not reduced, suggesting alternative glycan extension by ALG12 and ALG9 likely occurs in the majority of individuals with ALG3-CDG. Similar to $Man_9GlcNAc_2$, most individuals with ALG3-CDG have reduced $Man_8GlcNAc_2$. Since there is no known pathway that can extend glycans to $Man_8GlcNAc_2$ or $Man_9GlcNAc_2$ without ALG3, the variable degree of deficiency in $Man_8GlcNAc_2$ may represent different levels of residual ALG3 activities in these individuals.

4 | DISCUSSION

Our 10 subjects bring the total of reported ALG3-CDG individuals to 40 since its initial identification in 1995[12–14] including three affected fetuses described by Denecke et al[15] and

Bian et al[22] as shown in Supplementary Table 2. In addition to the previously described phenotype, our affected individuals have clinical findings that were not previously reported for ALG3-CDG. The new findings include endocrine abnormalities (panhypopituitarism, hypothyroidism, and adrenal insufficiency), immunodeficiency, mild aortic root dilatation, renal anomalies (nephromegaly, cystic kidneys, and duplex kidney), and NTDs further expand the phenotype. Most of the reported patients had intractable nonfebrile seizures with different semiology despite treatment with multiple antiepileptic drugs (AEDs) in addition to a ketogenic diet (Table 1). Most of our subjects were also on multiple AEDs, and one had a vagus nerve stimulator in addition to receiving AEDs. A wide array of AEDs was utilized in a small number of individuals so, unfortunately, we are unable to discern a pattern of drug efficacy. Our cohort has facial dysmorphism (Table 1; Supplementary Figure 1), without a consistent dysmorphology. The features reported include hypertelorism, broad flat nasal bridge, large ears and micrognathia, large, dysplastic low-set ears, and inverted hypoplastic nipples.

Protein hypoglycosylation impacts many organ systems and impacts protein-protein interactions, especially in receptor-ligand and cell-cell signaling.[29] Glycoproteins are essential for endocrine homeostasis and are involved with regulating growth, metabolism and sexual development.[30,31] Previously reported endocrine involvement in ALG3-CDG was limited to delayed menarche in two siblings,[16] hyperinsulinemic hypoglycemia in another subject[4] and another neonate with refractory hypoglycemia.[17] Our new cases highlight that endocrinopathy is a recurring feature of ALG3-CDG. Four of our subjects had central hypothyroidism with three requiring hormone replacement. Although clinical hypothyroidism is rare, biochemical thyroid abnormalities are common in individuals with CDG.[32] Additionally, two of our newly reported individuals have panhypopituitarism (hypothyroidism, growth hormone deficiency, and adrenal insufficiency); both are on hormone replacement therapy. Furthermore, genital anomalies were found in (2/10) subjects, including ambiguous genitalia and micropenis. Hypocholesterolemia occurred in some of our cases. Protein glycosylation can regulate lipid metabolism.[33] It has been speculated that hypoglycosylation of LDL facilitates hepatic uptake of cholesterol[31] and defective N-glycosylation increases cell surface LDL receptor, likely through increased SREBP2 (sterol regulatory element-binding protein 2) protein expression.[34]

The immunological aspect of glycosylation disorders has been recently a field of great interest as the function of many glycosylated immunologic proteins, including immunoglobulins, adhesion molecules, complements, cytokines, and immune receptors, is influenced by glycosylation.[35] Variable severity of immune dysfunction has been frequently observed in some CDG without clear immunological markers such as white blood cell or immunoglobulin alterations; however, it has not been reported clearly in ALG3-CDG.[35] Repeated pulmonary and ear infections have been reported in three individuals with ALG3-CDG.[5,12,18] Furthermore, fatal urosepsis occurred in a neonate with ALG3-CDG,[4] urinary tract infection in another infant,[2] pulmonary infection in a 22-months old female,[6] two episodes of pneumonia in the first year of life requiring hospitalization in the intensive care unit in one infant and a febrile illness with respiratory distress requiring hospitalization in the twin sibling.[14] In our cohort, recurrent infections have been observed in 6/8 (75%) individuals, but immunological studies were obtained in only two of them. Interestingly, it is

unclear if P3's less severe COVID-19 course compared to the course of her previous infections is a reflection of typically more mild disease in pediatric patients or her underlying glycosylation disorder, which could affect both SARS-CoV-2 spike protein glycosylation and ACE2 receptor glycosylation. Our study illustrates that while seldomly currently performed, immunologic evaluation is important in the management of ALG3-CDG patients.

Renal anomalies including hydronephrosis, hydroureters, pyelocaliceal dilatation, increased renal echogenicity, nephrocalcinosis, renal dysplasia, and ureteropelvic dysfunction have been previously reported in ALG3-CDG.[4,5,12,14,17,35] Renal involvement in several of our newly described individuals with ALG3-CDG highlights that structural renal involvement may be a feature of ALG3-CDG. Nephrocalcinosis has been seen in both P2 and P3; P3 has nephromegaly and cystic kidneys, while P6 had duplex kidney, findings that could reflect the pathophysiological impact of abnormal glycosylation on the kidneys. Cardiovascular involvement has been reported in 10 individuals with ALG3-CDG[4,11,12,14,17] as shown in Supplementary Table 2 and was noted in (6/8) of our subjects. Aortic root dilatation (Z score >2), with no other signs of connective tissue dysplasia, has been observed in four of our subjects, a finding that was not reported in prior cases with ALG3-CDG. Cardiomegaly and pulmonary valve dilatation, noted in P10, are additional new cardiovascular findings.

Our biochemical studies showed consistent increases of $Man_{0\text{-}4}GlcNAc_2$ and reduced $Man_9GlcNAc_2$ (Supplementary Table 1) with no presence of mannose deficient tetrasccharide, which differ from N-glycan profiles of PMM2-CDG; however, a variable reduction in $Man_8GlcNAc_2$ was similar to PMM2-CDG. A significant new biochemical finding was the marked reduction of N-linked hybrid glycans. This unique combination of plasma N-glycan changes can act as a specific diagnostic pattern for ALG3-CDG. While transferrin analysis is simple and a good first step, routine CDG workup should include ongoing plasma N-glycan profiling for confirmed cases. The degree of elevation of the mono/di oligo ratio of transferrin may correlate with severity and/or age, as seen in other CDGs, but we do not have enough power to definitively illustrate this.

NTDs were a shared phenotype between P2 and P3 (Table 1), who are distantly related, from the same small village in Ecuador and share the same inherited homozygous variant in *ALG3* c.796C>T [p.R266C]. This pathogenic variant was previously reported in a female with ALG3-CDG who was also homozygous for the variant, although due to de novo mutation and maternal segmental uniparental disomy (UPD) of chromosome 3 (q21.3-qter),[7] rather than consanguinity. P2 had prenatally diagnosed MM, and P3 was diagnosed with tethered cord after clinical evaluation of a sacral dimple. The MM was surgically repaired soon after birth. Similarly, the tethered cord required neurosurgical intervention after she developed progressive scoliosis requiring bracing which prompted proceeding with laminectomy for tethered cord release. P2 was identified on ES to be heterozygous for a paternally inherited variant of uncertain significance in *VANGL2*, a gene reported to be associated with increased risk for nonsyndromic NTDs with incomplete penetrance,[25,26] which was thought to possibly explain P2's MM. However, P3 does not carry the familial variant in *VANGL2,* making it a less likely explanation for their shared NTDs. NTD was not detected in the previously published individual with ALG3-CDG who shares the same

homozygous pathogenic variant.[7] It remains possible that NTD is a rare feature of ALG3-CDG.

Based on our observations, we could not find genotype-phenotype association among P2, P3, and Schollen et al's case with the same genetic variant (p.R266C). This could possibly be due to a phenotypic contribution from Schollen's case's segmental maternal UPD, although the individual's phenotypic severity is very similar to other reported individuals with ALG3-CDG suggesting that the individual's symptoms are entirely due to the homozygous *ALG3* mutation. On the other hand, we did notice a possible correlation with a severe phenotype for the previously reported homozygous variant p.R171Q[4,11] and shared by P5. All of these nine cases with this genotype passed away very early in life; P5 was stillborn, the individual reported by Sun et al died at age 19 days due to urosepsis,[4] while the remaining seven subjects reported by Alsubhi et al died in the neonatal period secondary to the complications of MCA.[11] We suspect that variants within TMD could alter protein orientation within the ER membrane, its stability, and donor or acceptor substrate binding. Without fibroblasts from all affected individuals, it is difficult to determine if a specific variant affects protein stability. Also, no confirmed crystal structure, substrate binding sites or catalytic active sites are available for ALG3, which hinders additional predictions. We saw no additional clear genotype-phenotype correlations. P5, P6, and P10, who all suffered peri/neonatal deaths, had variants in the cytoplasmic loops and in the TMDs. The variants that P8 and P9, with the mildest disease course, carried were both within TMDs (3 and 7). However, these two individuals were also siblings and these variants are currently private to this family. Thus, other factors such as genetic background and environment similarities preclude definitive genotype-phenotype correlation.

Based on previous cases and our novel findings, we recommend newly diagnosed patients undergo a thorough evaluation (Table 2) that includes developmental assessment with initiation of physical, occupational, and speech therapies; careful history to evaluate for seizures and to possibly include EEG; cardiology examination including echocardiogram; physical examination for skeletal anomalies; thorough ophthalmologic exam; nutrition and growth assessment including evaluation by licensed dietitian; and laboratory assessment for transaminitis, anemia, and coagulation abnormalities. Baseline evaluation should also include the endocrine system, specifically interrogation of the adrenal axis, thyroid function, and growth factors; renal evaluation should include renal ultrasound and urinalysis, and immunological evaluation at least evaluating history of recurrent infection, but possibly to also include response to vaccination and immunoglobulin levels.

In conclusion, we present 10 additional cases of ALG3-CDG with 11 novel variants, further clinical features and new biomarkers related to glycosylation dysfunction in ALG3-CDG. Our report further expands the clinical and molecular spectrum of ALG3-CDG, demonstrates unique plasma N-glycan patterns, and provides recommendations for management of individuals with ALG3-CDG.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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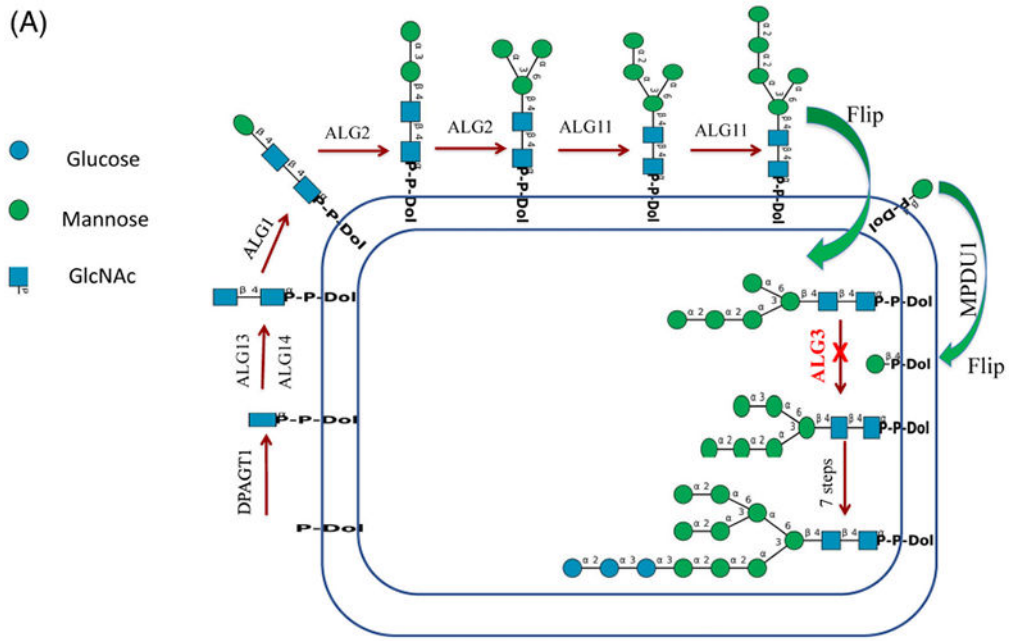

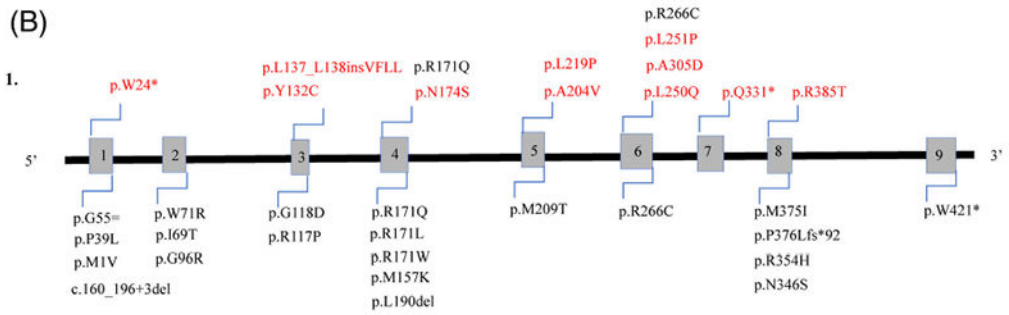

2.

p.W24	p.Y132	p.N174	p.A204	p.L219	p.L251	p.A305	p.L250	p.Q331	p.R385	Species
W	Y	N	A	L	L	A	L	Q	R	Human
W	Y	N	A	L	L	A	L	Q	R	Rhesus
W	Y	N	A	L	L	A	L	Q	R	Mouse
W	Y	N	A	L	L	A	L	Q	R	Dog
S	Y	N	A	L	L	A	L	Q	R	Elephant
G	Y	N	A	T	L	A	L	P	K	Chicken
-	Y	N	A	L	L	C	L	H	K	X_tropicalis
-	Y	N	A	L	L	A	L	H	R	Zebrafish

FIGURE 1.

Pathway showing the enzymatic step requiring ALG3 mannosyltransferase activity and schematic showing variants identified within *ALG3*. A, Schematic showing only the early N-linked glycosylation pathway on the cytoplasmic facing side of the endoplasmic reticulum with the ALG3-dependent step highlighted with a red (X) over the arrow. B, 1. Schematic showing exon location of all the *ALG3* variants identified in this study (upper part). The novel variants identified in this study are highlighted in red. The lower part of the figure represents all the *ALG3* variants reported in the literature. The variant c.296+4A>G has

been reported as well, but it is intronic (intron 2 of 8) and is not represented in the figure. 2. Multiple sequence alignments of all novel variants reported in this study are provided in the table

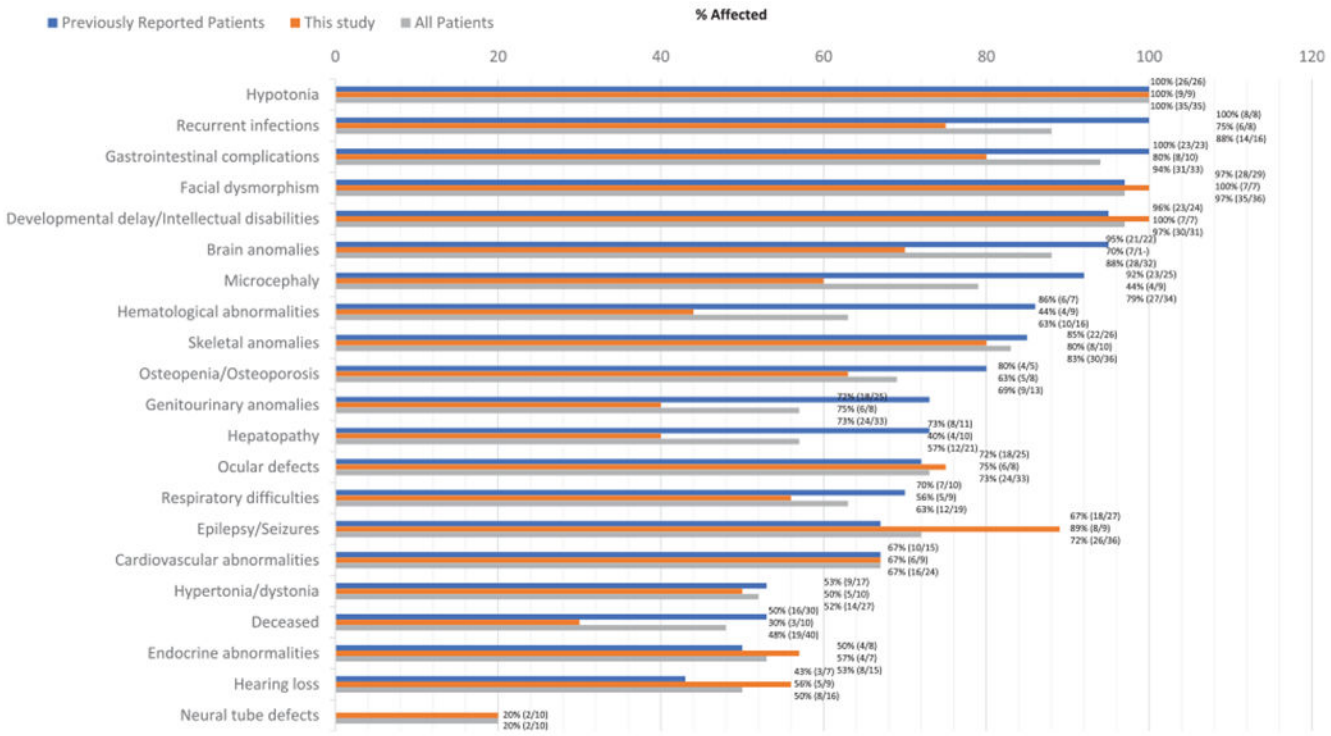

FIGURE 2.

Clinical summary of 30 previously reported individuals with ALG3-CDG (blue bars) along with our cohort (orange bars) and total number of individuals with ALG3-CDG including this study (gray bars)[2,4–18]

TABLE 1

Overview of ALG3-CDG individuals

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9	Individual 10
DNA variant[a]	c.656T>C/ c.749T>A	c.796C>T/ c.796C>T	c.796C>T/ c.796C>T	c.991C>T/ c.914C>A	c.512G>A/ c.512G>A	c.611C>T/ c.1154G>C	c.72G>A/ c.521A>G	c.395A>G/ c.752T>C	c.395A>G/ c.752T>C	c.410_411insTGTCTTCTTGCT/ c.410_411insTGTCTTCTTGCT
Protein variation	Leu219Pro/ Leu250Gln	Arg266Cys/ Arg266Cys	Arg266Cys/ Arg266Cys	Gln331Ter/ Ala305Asp	Arg171Gln/ Arg171Gln	Ala204Val/ Arg385Thr	Trp24Ter/ Asn174Ser	Tyr132Cys/ Leu251Pro	Tyr132Cys/ Leu251Pro	Leu137_Leu138insValPheLeuLeu/ Leu137_Leu138insValPheLeuLeu
Novel variant	Y/Y	N/N	N/N	Y/Y	N/N	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y
Variant classification[b]	Pathogenic/ pathogenic (PS3, PM2, PM3, PP2, PP3, GERP 5.26/ PS3, PM1, PM2, PP2, PP3, GERP 5.58)	Pathogenic/ pathogenic (PS3, PM1, PM2, PP2, PP3, GERP 5.58)	Pathogenic/ pathogenic (PS3, PM1, PM2, PP2, PP3, GERP 5.58)	Pathogenic/ pathogenic (PVS1, PS3, PM2, PP3, PP5, GERP 5.64/ PS3, PM2, PM3, PP2, PP3, GERP 5.78)	Likely pathogenic/ likely pathogenic (PM1, PM2, PP2, PP3, PP5, GERP 5.14)	Pathogenic/ pathogenic (PS3, PM2, PM3,PP2, PP3, GERP 5.26/ PVS1, PS3, PM2, PP2, PP3 GERP 5.09)	Pathogenic/ likely pathogenic (PVS1, PM2, PP3, GERP 5.6/PM1, PM2, PM3, PP2, PP3, GERP 5.14)	Pathogenic/ pathogenic (PS3, PM1, PM2, PM3, PP2, PP3, PP5, GERP 5.19/ PS3, PM1, PM2, PP2, PP3, PP5, GERP 5.58)	Pathogenic/ pathogenic (PS3, PM1, PM2, PM3, PP2, PP3, PP5, GERP 5.19/ PS3, PM1, PM2, PP2, PP3, PP5, GERP 5.58)	Likely pathogenic/likely pathogenic (PM1, PM2, PM4, PP3)
Inheritance	Inherited	Inherited	Inherited	Inherited/De novo	Inherited	Inherited	Inherited	Inherited	Inherited	Inherited
Age at diagnosis	16 years	13 mo	2 mo	4 years	Birth	ND	22 years	33 years	30 years	Antenatal (20 weeks)
Sex	F	M	F	M	F	M	F	M	F	F
Ethnicity	Caucasian	Ecuador	Ecuador	African American	Arabic	African American	Caucasian	Caucasian	Caucasian	Arabic-Saudi
Family history and consanguinity	-	Distant cousins	Distant cousins	-	Distant cousins	-	-	Affected sister	Affected brother	First cousins
Prenatal complications	Gestational diabetes, gallstone pancreatitis	Myelomeningocele	Fetal pyelectasis	-	Polyhydramnios, fetal akinesia	-	-	-	Hyperemesis	Hypothyroidism, IUGR
Microcephaly	− (36%)	+	+ (<1%)	+	+	−	−	−	−	+
Height/Weight (age) at time of microcephaly determination	4%/22% (17 years)	ND	50%/50% (2 years)	0.19%/2.6% (6 years)	ND	ND	Normal/ normal (birth)	60%/5% (33 years)	89%/62%	ND
GDD/ID	+	+	+	+	ND	ND	+	+	+	ND
Seizures	+	+	+	+	ND	+	+	+	+	−
Seizures semiology	Drop, GTC, Focal TC, Lennox-Gestaut, absence, nocturnal convulsive, tonic, myoclonic	Infantile spasms, GTC, myoclonic	Infantile spasms, GTC, myoclonic	Myoclonic	NA	NA	GTC, absence, simple partial and complex partial	GTC, atonic	GTC, atonic	NA
Epilepsy treatment	Clobazam, methsuximide, valproate, acetazolamide, felbamate, topiramate, lamotrigine,	Valproate, vigabatrin	Levetiracetam, topiramate	Clobazam, rufinamide	NA	NA	Phenobarbital, valproate, lamotrigine, carbamazepine	Felbamate	Phenobarbital, currently off AED	NA

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	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9	Individual 10
	clorazepate, levetiracetam, VNS									
Brain anomalies	Mild prominence of extra-axial spaces along parietooccipital lobes	Dysmorphic cerebral and cerebellar hemispheres, dysplastic CC, Chiari II deformity, gray matter heterotopia, enlarged dysmorphic supratentorial ventricles, deficient portions of anterior and posterior interhemispheric falx, polymicrogyria	Microcephaly, brain atrophy, thin brainstem and CC, delayed myelination, small optic nerves, chiasm, and tracts, abnormal internal auditory canals and shallow sella turcica without a normal neurohypophysis	Diffuse cerebral atrophy	-	Cerebellar atrophy/ dysplasia	Thin CC, generalized prominence of ventricles and sulci, mega cisterna magna, subarachnoid cyst	Prominent ventricles and sulci	Cavum septum pellucidum	Cerebellar atrophy/dysplasia
NTD	–	Myelomeningocele	Tethered cord	–	–	–	–	–	–	–
Hypotonia	+	+	+	+	ND	+	+	+	+	+
Hypertonia/ dystonia	–	–	–	–	+	+	+	+	+	–
Facial dysmorphism	+ Low sloping forehead, thick eyebrows/eyelashes, broad/flat nasal bridge, prominent mandible and maxilla with large-appearing mouth, thick lips, widely spaced teeth with mild hypertrophy of gum, high palate	+ Brachycephaly, triangular face, pointed chin, long nose with broad bulbous tip, hypoplastic alae nasi, columella extends below the nares, protruded low set cupped ears, simple helices, arched eyebrows, epicanthal folds, long eyelashes, telecanthus of the lids, thin lips, smooth philtrum, discolored dentition and widely spaced teeth	+ Plagiocephaly, coarse facial features, hypertelorism, arched eyebrows, bulbous nasal tip, long philtrum, epicanthal folds, low set protruding dysplastic ears, high palate	+ Hypertelorism, anteriorly attached earlobes	+ Down-slanting palpebral fissures	+ Hypertelorism	+ Epicanthal folds, broad depressed nasal bridge, bulbous nose, hypertelorism, large protruding ears, thin upper liP	+ Down-slanting palpebral fissures	ND	+ Flat/broad nasal bridge, micrognathia
Ophthalmological impairment	+ (strabismus, myopia)	+ (optic nerve atrophy)	+ (cortical blindness)	+ (strabismus, myopia, impaired visual awareness)	ND	+ (optic nerve atrophy)	+ (strabismus)	–	–	ND
Hearing loss	+	+	+	–	ND	+	–	+[c]	–	ND
Feeding difficulties	+	+	+	+	NA	+	+	+	+	ND
Hepatopathy	+	+	+	+	NA	–	–	–	–	–
Hypolipidemia	+	+	+	ND	ND	ND	ND	ND	ND	ND
Hematological abnormalities	Low FXI, ATIII	Low FXI, ATIII, prolonged APTT	Low ATIII, prolonged APTT	–	NA	–	Low ATIII	–	–	ND

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9	Individual 10
Cardiac abnormalities	–	–	Dilated aortic root, TR, sinus bradycardia	Dilated aortic root	ND	Dilated aortic root, ASD, PAH	Single coronary artery	Increased LV cavity size, AR	ND	Cardiomegaly, dilated aortic root, dilated AV, PV
Respiratory difficulties	–	OSA on CPAP	Bronchiectasis on oxygen	–	NA	On ventilator	–	Nocturnal apnea	–	Apnea
Skeletal findings	–	Arthrogryposis, hip sublaxation, hip dysplasia, hip dislocation, clubfeet, camptodactyly, contractures, scoliosis	Arthrogryposis, overlapping toes, cortical thumb, contractures, scoliosis	Arthrogryposis, overlapping toes, scoliosis, contractures	Overlapping toes, hand/finger abnormalities, contractures	Arthrogryposis, talipes, camptodactyly, contractures	Scoliosis	Scoliosis	–	Arthrogryposis, clenched hands, clubfoot, scoliosis
Osteopenia/ osteoporosis	–	+	ND	+	ND	+ (recurrent fractures)	+ fractures (pelvis, clavicle)	+ (fracture of T10)	–	–
Endocrine abnormalities	Panhypopituitarism, hypoglycemia	Panhypopituitarism, hypoglycemia	–	–	ND	ND	–	Hypothyroidism	Hypothyroidism	ND
Recurrent infections	+	+ weekly IVIG	+	+	NA	+	+	–	–	NA
Genitourinary anomalies	–	Nephrocalcinosis, neurogenic bladder, micropenis	Nephrocalcinosis, nephromegaly, cystic kidneys, non-obstructive stones	Phimosis	–	Duplicated kidney, ambiguous genitalia	–	–	–	Hydronephrosis
CDG testing -CDT: Mono/Di (ref value<0.06) -N-glycan -LLO	-CDT: type I, Mono/Di = 0.25 -N-glycan: increased Man0-4, reduced Man6-9 and hybrid glycan - LLO: accumulation of N_2M_5	-CDT: type I, Mono/Di = 0.66 (13 mo), 0.26 (30 mo), 0.23 (38 mo), 0.20 (62 mo), -N-glycan: increased Man0-4, reduced Man6-9	-CDT: type I, Mono/Di = 0.56 (9 mo), 0.52 (13 mo), 0.28 (17 mo), 0.20 (29 mo) -N-glycan: increased Man0-4, reduced Man6-9	-CDT: type I, Mono/Di = 0.48 -N-glycan: increased Man0-4, reduced Man8-9	ND	-CDT: type I, Mono/Di = 0.656 -LLO: accumulation of N_2M_5	-CDT: type I, Mono/Di = 0.168	-CDT: type I, Mono/Di = 0.13 -LLO: accumulation of N_2M_5	-CDT: type I, Mono/Di = 0.15 -LLO: accumulation of N_2M_5	ND
NPCRS	Severe (36) at 17 years	Severe (37) at 5 years	Severe (30) at 2 years	Severe (47) at 7 years	NA	ND	Severe (37) at 30 years	Moderate (25) at 37 years	Moderate (17) at 35 years	NA
Survival	Alive at 18 years	Alive at 6 years	Alive at 2 years	Alive at 7 years	Stillbirth	Died at 1 year	Alive at 30 years	Alive at 38 years	Alive at 36 years	Died after birth

Abbreviations: AED, antiepileptic drugs; APTT, activated prothrombin time; AR, aortic valve regurgitation; ASD, atrial septum defect; ATIII, antithrombin III; AV, aortic valve; CC, corpus callosum; CDT, carbohydrate deficient transferrin; CPAP, continuous positive airway pressure; Di, di-glycosylated transferrin; F, female; FXI, Factor XI; GTC, generalized tonic clonic; IUGR, intrauterine growth restriction; IVIG, intravenous immunoglobulin; LLO, lipid-linked oligosaccharide; LV, left ventricle; M, male; Man, mannose; mo, months; Mono, mono-glycosylated transferrin; N, no; NA, not applicable/not available; ND, not determined; NPCRS, Nijmegan pediatric CDG rating scale; NTD, neural tube defect; OSA, obstructive sleep apnea; PAH, pulmonary artery hypertension; T10, thoracic vertebra 10; TC, tonic-clonic; TR, tricuspid regurgitation; Y, yes; VNS, vagus nerve stimulator.

[a]The reference transcript is NM_005787.6.

[b]Criteria for classifying pathogenicity of variants per Richards et al. Genomic evolutionary rating profiling (GERP) and a simple conservation test that is used to establish whether the position is conserved. PVS1: Pathogenic, very strong (null variant [nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single, or multiexon deletion] in a gene where LOF is a known mechanism of disease); PS1: Pathogenic, strong (same amino acid change as a previously established pathogenic variant regardless of nucleotide change); PS3: Pathogenic, strong (well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product); PM1: Pathogenic, moderate (located in a mutational hot spot and/or critical and well-established functional domain [eg, active site of an enzyme] without benign variation); PM2: Pathogenic, moderate (absent from controls [or at extremely low frequency if recessive] in Exome Sequencing Project, 1000 Genomes Project, or Exome

Aggregation Consortium); PM3: Pathogenic, moderate (for recessive disorders, detected in trans with a pathogenic variant); PP2: Pathogenic, supporting (Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease); PP3: Pathogenic, supporting (multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.); PP5: Pathogenic, supporting (reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation).

[c]Individual 8 with hearing loss, was also heterozygous for R1494X pathogenic variant in *LOXHD1* associated with recessive hearing loss and Fuchs corneal dystrophy. Individual 8 does not have corneal dystrophy. This variant also in his sister (individual 9) and father neither of whom had hearing loss or corneal dystrophy. Direct sequencing/deletion analysis in individual 8 did not identify a second variant in *LOXHD1*. Contribution to hearing loss in individual 8 cannot be ruled out.

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TABLE 2

Suggested guidelines for evaluation and follow-up of individuals diagnosed with ALG3-CDG

			First year of life	From 1 to 2 years of age		
Evaluation		**Baseline evaluation at diagnosis**	**Every 6 months**	**Every 6 months**	**Yearly**	**After 2 years of age**
CDG testing	CDT	X	X	X		Yearly[a]
	N-glycan	X				
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]
	Liver US	X[a]				
Hematological evaluation	CBC	X	X[a]	X[a]		Yearly[a]
	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]
	Blood glucose	X	X[a]		X[a]	
Immunological evaluation	Hx of recurrent infections	X	X	X		Yearly
	Immunoglobulins	X[a]				
	Response to vaccines	X				After each vaccine series

Evaluation		Baseline evaluation at diagnosis	First year of life	From 1 to 2 years of age		After 2 years of age
			Every 6 months	Every 6 months	Yearly	
Cardiology evaluation	Echo	X[a]			X[a]	Every 5 years[a]
Neurological evaluation	Brain MRI	X[a]				PRN
	EEG	X[a]				PRN
Development Assessment	PHT/OT/ST	X	X	X		Yearly[a]
Ophthalmological evaluation		X			X[a]	Yearly[a]
Physical exam for skeletal anomalies		X				
Nutrition and Growth Assessment		X	X[a]	X[a]		Yearly[a]

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; ATIII, anti-thrombin III; CBC, complete blood count; CDT, carbohydrate deficient transferrin; Echo, echocardiogram; EEG, electroencephalogram; FXI, factor XI; Hx, history; Igs, immunoglobulins; INR, international normalized ratio; LLO, lipid-linked oligosaccharides; MRI, magnetic resonance imaging; OT, occupational therapy; PHT, physical therapy; PRN, pro re nata (as needed); 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.