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ALG13 X-linked intellectual disability: New variants, glycosylation analysis, and expanded phenotypes

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AUTHOR CONTRIBUTIONS

Hind Alsharhan provided clinical evaluations, drafted the initial manuscript, and revised the manuscript. Miao He, Andrew C. Edmondson, Earnest J. P. Daniel, Jie Chen, Tyhiesia Donald, Somayeh Bakhtiari, David Amor, Elizabeth A. Jones, Grace Vassallo, Marie Vincent, Benjamin Cogné, Wallid Deb, Arend H. Werners, Sheng C. Jin, Kaya Bilguvar, John Christodoulou, Richard I. Webster, Katherine R. Yearwood, Bobby G. Ng, Hudson H. Freeze, Michael C. Kruer, Dong Li, Andrew K. Sobering, Kimiyo M. Raymond, and Elizabeth J. Bhoj provided clinical evaluations, critically reviewed, and revised the manuscript. Miao He provided N-glycan analysis and interpretation, critically reviewed, and revised the manuscript.

Hind Alsharhan and Miao He contributed equally to this work.

CONFLICT OF INTEREST

H. H. F. is a consultant for Cerecor, Inc. The other authors declare that they have no conflicts of interest.

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. Informed consent was obtained from the parents of all patients for being included in the study. Additional informed consent was obtained from the parents of all patients for which identifying information is included in this article. The institutional review board of the Children's Hospital of Philadelphia approved this study.

ANIMAL RIGHTS

This article does not contain any studies on 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

Pathogenic variants in *ALG13* (*ALG13 UDP-N-acetylglucosaminyltransferase subunit*) cause an X-linked congenital disorder of glycosylation (ALG13-CDG) where individuals have variable clinical phenotypes that include developmental delay, intellectual disability, infantile spasms, and epileptic encephalopathy. Girls with a recurrent de novo c.3013C>T; p.(Asn107Ser) variant have normal transferrin glycosylation. Using a highly sensitive, semi-quantitative flow injection-electrospray ionization-quadrupole time-of-flight mass spectrometry (ESI-QTOF/MS) N-glycan assay, we report subtle abnormalities in N-glycans that normally account for <0.3% of the total plasma glycans that may increase up to 0.5% in females with the p.(Asn107Ser) variant.

Among our 11 unrelated ALG13-CDG individuals, one male had abnormal serum transferrin glycosylation. We describe seven previously unreported subjects including three novel variants in *ALG13* and report a milder neurodevelopmental course. We also summarize the molecular, biochemical, and clinical data for the 53 previously reported ALG13-CDG individuals. We provide evidence that *ALG13* pathogenic variants may mildly alter N-linked protein glycosylation in both female and male subjects, but the underlying mechanism remains unclear.

Keywords

carbohydrate deficient transferrin; congenital disorders of glycosylation; epilepsy; exome sequencing; mass spectrometry; N-glycans

1 | INTRODUCTION

Congenital disorder(s) of glycosylation (CDG) comprise a rapidly expanding group of over 140 diseases in protein and/or lipid glycosylation.[1,2,3] These multisystem disorders are both clinically and genetically heterogeneous. Glycosylation is the process of adding sugar residues (glycans) to proteins and lipids in different cellular pathways mainly in the endoplasmic reticulum (ER) and Golgi apparatus. Pathogenic variants in *ALG13* (encoding UDP-N-acetylglucosaminyltransferase subunit) were first reported as an X-linked cause of congenital disorders of glycosylation type 1 (ALG13-CDG) and as a cause of X-linked intellectual disability (XLID).[4–6]

Variants in *ALG13* are associated with variable clinical phenotypes, mainly consisting of developmental delay (DD), intellectual disability (ID) and epileptic encephalopathy (EE). *ALG13* is located on Xq23 and its encoded protein forms a heteromeric ALG13/ALG14 complex in the ER.[4,7] This complex functions as a UDP-N acetylglucosamine (GlcNAc) transferase used for the second step of protein N-glycosylation by extending $GlcNAc_1$-PP-dolichol to $GlcNAc_2$-PP-dolichol, prior to assembly of high mannose N-glycans.[8,9] Pathogenic *ALG13* variants would be expected to result in glycosylation pattern abnormalities, with unoccupied glycosylation sequons, but nearly all reported individuals with ALG13-CDG have essentially normal glycosylation. This highlights the need for additional glycoprotein biomarkers and emphasizes the importance of genetic testing for the diagnosis of ALG13-CDG.

A literature search identifies 53 individuals with variants in *ALG13*, the most frequent being a de novo c.320A>G; p.(Asn107Ser) variant in 37 females and two males with DD, infantile spasms (IS)/West syndrome, and EE. Here, we report an additional 11 unrelated individuals including three inherited, novel variants in *ALG13.* We expand the phenotypic spectrum of ALG13-CDG and describe a milder phenotype in affected individuals. Further, using a semi-quantitative, expanded plasma N-glycan profiling assay with increased sensitivity, we studied nine ALG13-CDG individuals, including four previously reported cases[10] and observed very mild, though consistent, glycosylation abnormalities among female individuals with known pathogenic variants in *ALG13.* Additionally, we review the 53 previously reported individuals with *ALG13* variants to summarize our knowledge of this rare CDG.

2 | MATERIALS AND METHODS

2.1 | Individuals

Three individuals of our cohort were identified through GeneMatcher.[10] Written informed consent was obtained from the parents of the 11 individuals for study participation and publication of photographs. Individuals 7 (CDG-0456), 8 (CDG-0136), 10 (CDG-0417) and 11 (CDG-0431) were previously reported by Ng et al.[11] A PubMed database search was performed using the terms: ALG13 and CDG. All publications were included in the search from the time of the disorder discovery.

2.2 | Clinical studies

To assess intermediate glycans from the N-linked protein glycosylation pathway, we measured the semi-quantitative plasma N-glycan profiles from two male and seven female patients (individuals 1, 2, 5, 6, 7, 8, 9, 10 and 11) with a previously reported clinically validated semi-quantitative N-glycan assay[12] using flow injection-electrospray ionization-quadrupole time-of-flight mass spectrometry (ESI-QTOF/MS). Briefly, heparinized plasma was combined with an internal control (sialylglycopeptide) and digested in buffered RapiGest SFTM solution. N-glycans were cleaved with PNGase FTM, reacted with RapiFluor-MS, and isolated on a HILIC column. Mass spectrometric analysis was performed on the Waters' Synapt G2 Si QTOF in positive ion mode as previously described.[12] We calculated the difference between the observed means in two independent samples using an online tool: https://www.medcalc.org/calc/comparison_of_means.php. A significance value (*P*-value) and 95% Confidence Interval (CI) of the difference is reported. We used this assay to evaluate 53 unique N-glycans. All the glycan ions evaluated had the fragment of the derivation tag and known glycan fragment ions. Reference values were derived from a previously obtained cohort of 115 normal/non-CDG controls.

Carbohydrate transferrin testing (CDT) was performed using a mass spectrometry approach (LC-ESI-TOF/MS)[13,14] for the nine individuals tested. Serum transferrin isoelectric focusing (TIEF) was carried out for individuals 3 and 4, as described.[15] *ALG13* variants were identified with exome sequencing (ES); individual 4 was diagnosed via the Deciphering Developmental Disorders (DDD) Study[16] and individual 11 was identified via genome sequencing. No other definitive genetic diagnosis was identified in any of these individuals.

3 | RESULTS

3.1 | Clinical findings

Summary of the clinical, biochemical, and genetic data of the 11 individuals in this study is presented in Table 1. A summary of the previously reported subjects with ALG13-CDG is shown in Table 2. Detailed descriptions and a clinical synopsis of each of the 11 individuals are provided in the supplementary material. The three males and eight females ranged from 7 months to 13 years; all are alive. All (except individual 3) had some degree of neurodevelopmental abnormalities including DD and/or ID (10/11), seizures/epilepsy (8/11), hypotonia (6/11), microcephaly (6/11), and abnormal brain imaging (5/9) which consisted primarily of brain atrophy and benign prominence of subarachnoid space.

Ocular abnormalities were reported (3/11) and mainly involved strabismus (1/3), myopia (1/3) and cortical visual impairment (2/3). Sensorineural hearing loss was uncommon (1/11). Facial dysmorphism was reported in four individuals and consisted mainly of prominent forehead, bulbous nose, large mouth and ears, prominent mandible, smooth philtrum, thin upper lip, large ears, widely spaced teeth, and high palate. Dysmorphism was not observed in the three male individuals. Facial images of two females and two males are shown in Figure S1. Gastrointestinal problems were observed in three individuals, and included feeding difficulties (3/3), tube feeding (3/3), reflux (1/3) and Crohn's disease (1/3).

3.2 | Variant analysis

Most of the previously reported variants are de novo, but we identified three inherited novel variants as shown in Table 1 and predicted their likely pathogenicity using Varsome[17] together with analysis of glycosylation for individuals 1 and 2. The *ALG13* c.3013C>T; p.(Pro1005Ser) variant in individual 1 is classified as likely pathogenic and damaging by five out of eight different computational programs and its absence in gnomAD. Transferrin analysis was also mildly abnormal. The c.2458-15_2486del variant in individual 2 is classified as pathogenic because it is predicted to affect a splice junction in the *ALG13* pre-mRNA and is associated with an abnormal transferrin glycosylation profile. We were unable to classify the pathogenicity of the *ALG13* c.2272G>T; p.(Val758Phe) variant observed in individual 3, since a sample was not available for glycosylation analysis. However, it was predicted to be damaging by six out of nine different computational programs and is not present in gnomAD. Therefore, it was classified as a variant of uncertain significance. Known pathogenic de novo variants were identified in the eight female patients, four of which were previously described. The c.2915G>T; p.(Gly972Val) variant was previously described in a male individual.[11]

3.3 | Plasma N-glycan profiles in ALG13-CDG

Variable, mild increases in a series of small high mannose glycans were detected in both male and female subjects (nine individuals were tested: 1, 2, 5, 6, 7, 8, 9, 10 and 11) as shown in Figure 1A, and Table S1. $GlcNAc_2Man_1$ (Man1) or possibly $GlcNAc_2Gal_1$, (Gal1) was either increased, or at the upper end of the normal limits in all females who were tested (4 were increased, and 3 were at the high end of normal). The Man1 or Gal1 abundance between all ALG13-CDG individuals is significantly increased ($P < .001$) when compared with 115 controls. Unexpectedly, the abundance of N-linked $GlcNAc_2Man_0$, (an intermediate glycan associated with the product of the reaction that ALG13 catalyzes) was not reduced in either male or female individuals. Instead, it was either mildly increased (2/9) and above the mean of normal consisting of 0.04% of the total N-glycans (9/9) when compared to the mean of normal controls ($P < .0001$). Similarly, another downstream intermediate, $GlcNAc_2Man_2$, was also at the upper end of the normal range, and above the mean of normal controls with $P < .0001$. The N-linked mannose-deprived tetrasaccharide was also very slightly increased in two female ALG13-CDG individuals (2/9) with $P < 0.001$, detected in plasma N-glycans, but not on transferrin. Although mild, these changes are statistically significant. Taken together, they suggest a small, generalized suppression of early steps of high mannose glycan synthesis rather than assembly of dolichol pyrophosphate-linked $GlcNAc_2$.

3.4 | Plasma carbohydrate deficient transferrin profile

We analyzed plasma CDT profiles as a surrogate of N-linked protein glycosylation as shown in Table S2. Significantly increased mono-glycosylated transferrin species was detected in the plasma only from individual 2 (male). The mono/di-glycosylated transferrin ratio was 0.27 (normal < 0.05), consistent with a reduction in the occupancy of glycans on transferrin, as a typical type 1 pattern. In addition, minor glycoforms of transferrin including those with one normal glycan and one Man0, or Man1, or Man2 were also detected (Figure 1B). However, the type 1 pattern, nor the minor glycoforms seen in individual 2 were not detected in any females. Only one female (1/7) showed a borderline increase of the mono/di-glycosylated transferrin ratio and another female (1/7) showed a mild increase of transferrin glycoform containing one Man1 glycan. Interestingly, mild hypogalactosylation in transferrin was detected in 5 of the 7 females.

4 | DISCUSSION

ALG13-CDG was first reported in a male with refractory epilepsy, multiple congenital anomalies, and a serum Tf IEF type 1 pattern.[4] He died at the age of 1 year. A total of 53 individuals with ALG13-CDG are known (Table 2). Among the pathogenic alleles, the de novo c.320A>G; p.(Asn107Ser) (rs398122394) variant is by far the most frequent in heterozygous symptomatic females. They typically appear normal at birth, but develop early onset seizures, usually starting as infantile spasms (IS) that subsequently evolves into other mixed seizure types including Lennox-Gastaut syndrome suggesting that the impact of the variant does not manifest until after birth. Most individuals initially respond to adrenocorticotropic hormone (ACTH) therapy, but usually continue to suffer from epileptic seizures.[11,18–23] This was observed in individual 6 who responded initially to ACTH before seizures worsened again. Improved responsiveness with longer ACTH therapy has been reported.[24] Sometimes seizures respond to antiepileptic drugs or a ketogenic diet,[11,23,25] but many reported patients have seizures refractory to all interventions. In this study, individuals 7, 8, 9, 10 and 11 were all started on a ketogenic diet after which their seizures were better controlled.

Some individuals develop a hyperkinetic movement disorder described as “dyskinesia” or “choreoathetoid movements.”[4,19,21,26,27] Vigabatrin has been reported to increase the spasms and aggravate the movement disorder[11,19,25] and may induce reversible changes on brain MRI diffusion restriction in the thalamus and globus pallidus.[11] However, it has been effective in controlling the seizures in five reported individuals.[11] The IS in individual 6 was treated initially with vigabatrin that was soon discontinued and switched to ACTH. A vagus nerve stimulator was reported to be effective in only one individual.[11]

All previously reported individuals have severe DD, ID and hypotonia. They may have developmental regression, visual disturbances and feeding difficulties requiring a gastrostomy tube. Brain anomalies have been reported in the majority of patients (58%), and include cerebral atrophy, corpus callosum anomalies and enlargement of subarachnoid spaces.[6,11,18,20–22,25,26,28–31]

Individuals with the recurrent c.320A>G; p. (Asn107Ser) variant typically have normal serum glycoprotein levels and rarely have abnormal coagulation profiles. This variant was found de novo in 37 unrelated symptomatic girls, but only in two affected males whose phenotypes were similar to those of the affected females,[11,26] ruling out its complete lethality in males as was initially thought. It is puzzling why this recurrent de novo variant results in severe neurological phenotype in females. Possible explanations include: (a) skewed X-inactivation limited to the central nervous system[23]; (b) a dominant negative effect resulting in reduced ALG13 activity[26]; (c) differential expression or different tissue enzymatic requirements[23]; (d) failure to have a consistent glycosylation defect pattern in all individuals could also be explained by milder glycosylation impairment with higher residual enzyme activity or an overall lower flux through the glycosylation pathway[28]; or (e) this CDG escapes detection by the usual laboratory evaluation.[23] Any of these hypotheses could also be true for the other pathogenic *ALG13* variants, as the exact mechanism of the pathology is still unknown.

Small, incomplete N-glycans including $GlcNAc_2$, $GlcNAc_2Man_{1\text{-}2}$, and mannose deprived tetrasaccharide have been previously described by our group in different CDG patients.[12] Although the combined abundance of these minor glycans are approximately 0.3% of total glycans in normal human plasma, they may be useful to help further characterize a few CDG types. Our study supports the hypothesis that *ALG13* pathogenic variants, in some males and most of the females, do not block the assembly of these intermediates nor of the completed glycans. Instead, the production of these small intermediates appears slightly amplified in several individuals, in particular, females with known pathological variants. However, overall N-glycan assembly was found to be reduced on transferrin, a hepatic protein, in the male individual with the c.2458-15_2486del variant. Thus, it is conceivable that *ALG13* may encode a subunit with a regulatory function. So, either loss of function or gain of function variants may lead to disease. An alternative explanation is the possible existence of an ALG13-independent minor pathway to make truncated glycans, such as $GlcNAc_2$ and the mannose-deprived tetrasaccharide, but it is insufficient to compensate for the impact of ALG13 deficiency in the canonical pathway.

The main reported features in individuals with ALG13-CDG are early onset seizures, IS (West syndrome), epileptic encephalopathy (EE), and global DD or developmental regression. A recent study explored the possible molecular mechanisms of ALG13-related epilepsy by showing hyperactive mechanistic target of rapamycin (mTOR) signaling pathways in the cortex and hippocampus of *Alg13* knockout mice,[32] a model that disrupts a longer isoform of *Alg13*, but would be predicted to leave a shorter isoform containing exons essential for glycosylation intact. *Alg13* was expressed selectively in neurons but barely detected in astrocytes or oligodendrocytes, indicating a cell-type specific expression pattern. *Alg13* deficiency significantly increased susceptibility to seizures and aggravated their severity, suggesting that this gene provides a protective factor for epilepsy and is a potential target of seizure suppression. Further studies in these mice suggest that ALG13 may regulate $GABA_A$ receptor function.[33] However, there were no obvious glycosylation defects detected in this mouse model.

It is interesting that all carrier females in the familial cases, whose sons are affected, are clinically thought to be asymptomatic. Typically, patients with ALG13-CDG present with a severe neurological phenotype, however, the three affected male individuals we present, in addition to one reported male by Gadomski et al[28] have a milder phenotype with less severe cognitive defects (individual 1 and 2), and relatively normal cognition (individual 3) and normal TIEF. By N-glycan testing, the female carrier of the p.(Gly972Val) variant is the only female who had normal Man1 abundance. Further, all the males in our cohort do not have any type of seizures. Of the 10 male individuals reported in the literature who were tested for glycosylation, three had abnormal glycosylation studies (Table 2). One male had abnormal TIEF with severely reduced GlcNAc-transferase enzyme activity at 17%,[4] whilst another male had normal TIEF, but slightly increased monoglycosylated transferrin as revealed by MS[26]. The third affected male[28] had normal TIEF but abnormal cellular protein glycosylation as evidenced by the decreased fibroblast expression of the intercellular adhesion molecule 1 (ICAM-1), which is a cell-surface glycoprotein and a reported hypoglycosylation marker in cultured cells affected with CDG. In cultured fibroblasts treated with galactose, increased ICAM-1 expression was observed suggesting a potential dietary treatment for ALG13-CDG.[28] The pathogenicity of their mutations was confirmed in one male by the severely reduced activity of the GlcNAc-transferase enzyme activity in their cultured fibroblasts.[4,28] Interestingly, our study detected mild or intermittent hypogalactosylation in ALG13-CDG females, suggesting there may be a mild galactosylation defect shared among female individuals. Given the lack of universal glycosylation defect biomarkers for ALG13-CDG, model organism knockout/knockdown, enzymatic testing, and possibly CSF glycosylation studies could be considered to further understand the pathophysiology of this disorder.[23]

5 | CONCLUSION

Our study provides evidence that *ALG13* pathogenic variants can mildly alter N-linked protein glycosylation in both female and male individuals, although changes in these glycans are not unique to ALG13-CDG and therefore not diagnostic. The underlying mechanism remains unclear. These data enhance our understanding of the phenotypic heterogeneity caused by pathogenic variants in *ALG13.* Our study shows progress towards the development of more sensitive biochemical biomarkers for testing the glycosylation defect of ALG13-CDG. A better understanding of the increases of these small N-glycans will be essential to uncover the link between the ALG13 pathogenic variants and their phenotypic presentation.[34]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SYNOPSIS

We expand the genotype and phenotype of ALG13-CDG, find novel variants, and show subtle abnormalities of a few minor glycans in females with the recurrent de novo p.(Asn107Ser) variant.

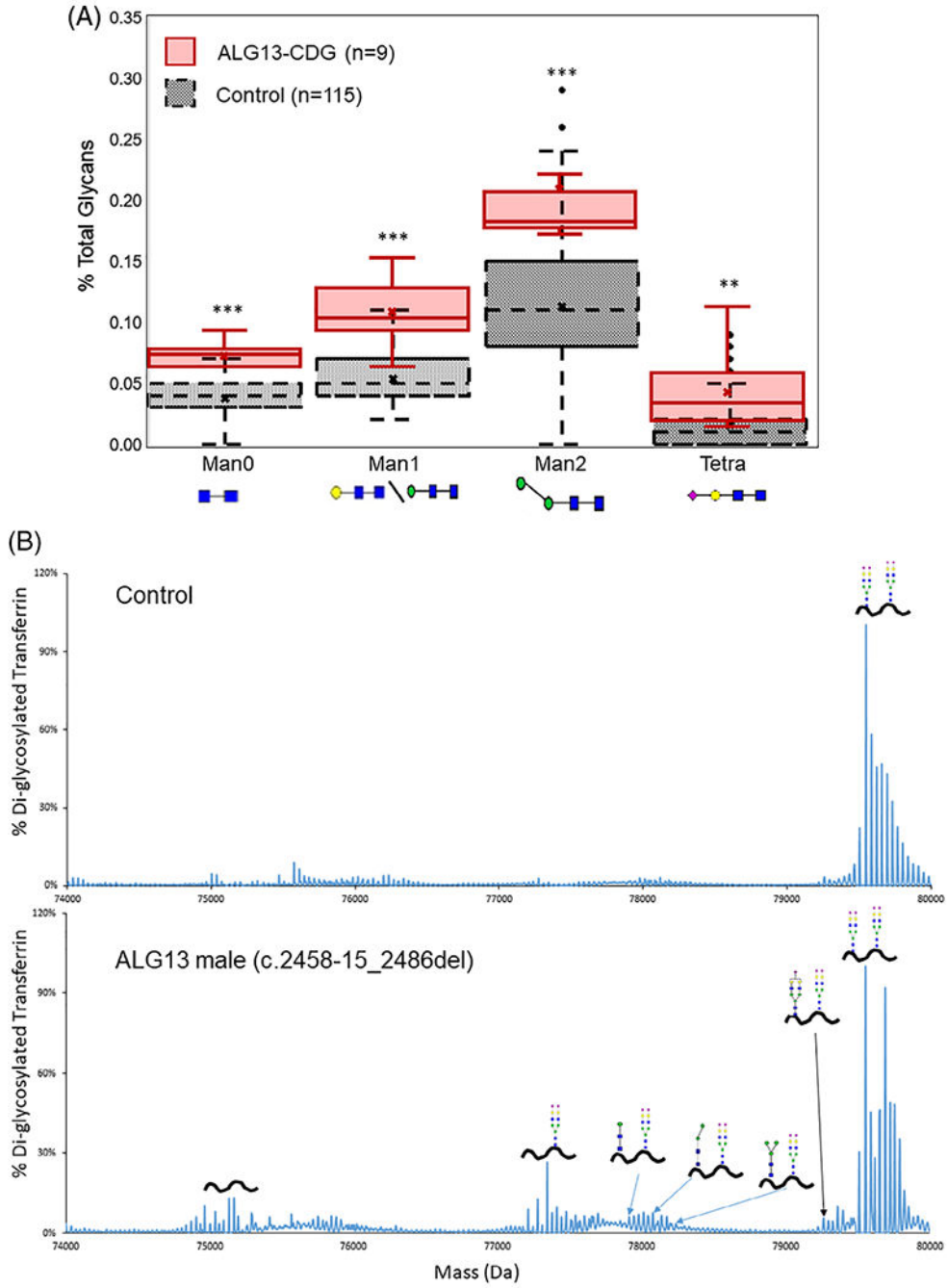

FIGURE 1.

The representative glycosylation changes identified in the plasma from ALG13-CDG individuals. A, Comparison between the abundance (% total N-glycan) of minor N-glycan species including N-linked $GlcNAc_2$ (Man0), $GlcNAc_2Gal_1$ or Man1, $GlcNAc_2Man_2$ (Man2), and $GlcNAc_2Gal_1NeuAc_1$(Tetra) in 9 ALG13-CDG patients (in red) and normal controls (in black). Data sets are shown as box and whisker plots with x showing the medium and outliers shown as dots. *** shows significance with $P < .0001$; ** shows significance with $P < .001$. B, The isotope envelopes of different plasma transferrin

glycoforms from a representative control and a male ALG13-CDG patient. The relative abundance of isotope envelopes of transferrin glycoforms are shown. Marked increases of mono-glycosylated transferrin glycoform at 26.5% and a-glycosylated transferrin at 13% of normal di-glycosylated transferrin glycoform were detected. Mild increases of mono-glycosylated transferrin with one $Man_1GlcNAc_2$ or $Man_2GlcNAc_2$ or $Man_3GlcNAc_2$ are also shown with blue arrows. Trisialo-transferrin is shown by a black arrow with essentially normal abundance

TABLE 1

Overview of the 11 individuals with ALG13-CDG described in this study

	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind. 6	Ind. 7 (CDG-0456)	Ind. 8 (CDG-0136)	Ind. 9	Ind. 10 (CDG-0417)	Ind. 11 (CDG-0431)
DNA var	c.3013C>T	c.2458-15_2486del	c.2272G>T	c.320A>G	c.320A>G	c.320A>G	c.320A>G	c.320A>G	c.2915G>T	c.241G>A	c.320A>G
Protein var	p.Pro1005Ser	—	p.Val758Phe	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Gly972Val	p.Ala81Thr	p.Asn107Ser
Novel var	Yes	Yes	Yes	No	No	No	No	No	No	No	No
Inheritance	Maternal	Maternal	Maternal	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo
Sex	Male	Male	Male	Female	Female	Female	Female	Female	Female	Female	Female
Ancestry	Afro-Caribbean	Afro-Caribbean	ND	Bangladeshi	French	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
Microceph	—	—	Yes	Yes	Yes	Yes	—	—	—	Yes	Yes
GDD/ID	Yes	Yes	—	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Seizures	—	—	—	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Brain anomalies	ND	ND	—	—	—	BESS	BESS	PVL	—	Progressive atrophy	Mild cerebral atrophy
Hypotonia	—	—	—	Yes	Yes	Yes	—	Yes	Yes	—	Yes
Regression	Yes	Yes	—	—	—	—	Yes	—	Yes	—	—
Facial dysmorph	—	—	—	—	Yes, see Figure S1	Yes, see Figure S1	—	—	—	Prominent forehead	High Palate
Ophthalm. impairment	—	—	—	—	—	Cortical blindness	Cortical blindness	Exotropia, amblyopia, myopia, astigmatism	—	—	—
SNHL	—	—	ND	ND	ND	—	Yes	—	—	—	—
Feeding difficulties	—	—	—	G-tube dependent	Reflux, G-tube dependent	—	—	—	—	—	G-tube dependent
Skeletal findings	—	Postaxial polydactyly	—	—	—	—	—	—	—	Vertebral anomalies, hemivertebrae	Severe generalized osteopenia
TIEF testing	ND	ND	Normal	Normal	ND	ND	ND	ND	ND	ND	ND
CDG testing CDT (MS)	Normal	Type I pattern	—	—	Borderline type I pattern	Mild under galactosylation	Mild under galactosylation	Normal	Mild under galactosylation	Mild under galactosylation	Mild under galactosylation

J Inherit Metab Dis. Author manuscript; available in PMC 2022 January 02.

	Ind. 1	Ind. 2	Ind. 3	Ind. 4	Ind. 5	Ind. 6	Ind. 7 (CDG-0456)	Ind. 8 (CDG-0136)	Ind. 9	Ind. 10 (CDG-0417)	Ind. 11 (CDG-0431)
CDG testing (N-glycan)	Normal	Normal	—	—	Moderate changes	Moderate intermittent changes	Mild changes	Very mild changes	Normal	Mild changes	Very mild changes
Other findings	—	—	—	Extra-pyramidal/ choreoathetoid movements	Sleeping disorder, Crohn's disease	Mitral valve regurgitation	Liver hemangioma, hepatomegaly, eczema	—	—	—	—
Survival	Alive, 6 years	Alive, 8 years	Alive, 13 years	Alive, 7 years	Alive, 12 years	Alive, 10 years	Alive, 4 years	Alive, 20 months	Alive, 7 months	Alive, 11 years	Alive, 15 years

Note: Summary of ALG13-CDG individuals.

Abbreviations: BESS, benign enlargement of subarachnoid space; CDT (MS), carbohydrate deficient transferrin via mass spectrometry; GDD, global developmental delay; G-tube, gastrostomy tube; ID, intellectual disabilities; ND, not determined; NPCRS, Nijmegan pediatric CDG rating scale; PVL, periventricular leukomalacia; SNHL, sensorineural hearing loss; TIEF, transferrin isoelectric focusing.

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TABLE 2

Overview of the 53 ALG13-CDG individuals reported in literature

	Timal 2012	Ligt 2012	Bissar-Tadmouri 2013 (4pts)	Epi4K 2013 Consortium (Allen) (2pts)	Michaud 2014	Hino-Fukuyo 2015	Smith-Packard 2015	Dimasi 2016	Epi4K 2016 Consortium (Myers)	Mooler 2016	Fung 2017	Galama 2017
DNA var	c.280A>G	c.320A>G	c.3221A>G	c.320A>G	c.320A>G	c.880C>T	c.320A>G	c.320A>G	c.320A>G	c.1641A>T	c.320A>G	c.320A>G
Protein var	p.Lys94Asp	p.Asn107Ser	p.Tyr1074Cys	p.Asn107Ser	p.Pro294Ser	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Gln547His	p.Asn107Ser	p.Asn107Ser	
Novel Var	Yes	No	Yes	No	No	Yes	No	No	No	Yes	No	No
Inheritance	De novo	De novo	Maternal	De novo	De novo	Maternal	De novo	ND	ND	Maternal	ND	De novo
Sex	Male	Female	Male (4)	Female	Female	Male	Female	Female	Female	Male	Female	Male
Ancestry	ND	ND	Arabic	ND	Caucasian	ND	ND	ND	ND	ND	Chinese	ND
Microceph	Yes	—	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
GDD/ID	ND	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	ND	Yes	Yes
Seizures	Yes	Yes	—	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Brain anomalies	ND	Cisterna magna, hydroceph, myelination delay, wide sulci	—	Slight prominence subarachnoid spaces (1 pt)	Cortical atrophy	CC anomaly	ND	Mild global atrophy	ND	ND	ND	CC hypoplasia, mild delay in myelination
Hypotonia	—	Yes	ND	ND	ND	ND	Yes	Yes	Yes	ND	—	Yes
Regression	—	—	ND	—	+	—	+	—	—	ND	—	—
Facial dysmorph	ND	Yes	ND	ND	ND	ND	ND	Yes	ND	ND	ND	Yes
Opthalm. impairment	Yes	Yes	ND	ND	ND	Yes	Yes	Yes	ND	ND	Yes	Yes
Feeding difficulties	ND	Yes	ND	ND	ND	ND	Yes	ND	ND	ND	ND	ND

	Timal 2012	Ligt 2012	Bissar-Tadmouri 2013 (4pts)	Epi4K 2013 Consortium (Allen) (2pts)	Michaud 2014	Hino-Fukuyo 2015	Smith-Packard 2015	Dimasi 2016	Epi4K 2016 Consortium (Myers)	Mooler 2016	Fung 2017	Galama 2017
Skeletal findings	Yes	Yes	ND	ND	ND	ND	ND	Yes	ND	ND	ND	Yes
CDG Testing	TIEF: abnormal; LLO: normal; GlcNAc transferase activity: low	ND	ND	ND	ND	ND	TIEF: normal	TIEF: normal	ND	ND	TIEF: normal	TIEF: normal; CDT (MS): lack of one glycan
Survival	Died, 1 year	Alive, 10 years	Alive, 6-15 years	ND	Alive, 7 years	Alive as adult	Alive, 7 years	Alive, 6 years	ND	ND	Alive, 2 years	Alive, 15 months
Other	Extrapyramidal symptoms, hepatomegaly, recurrent infections, prolonged APTT	Sleep disturbance, self-mutilation	—	—	—	—	—	—	Chorea	—	Dystonia	Chorea
	Gadomski 2017	**Hamici 2017**	**DDD 2017 (2 Pts)**	**Kobayashi 2017**	**Ortega-Moreno 2017**	**Bastaki 2018**	**Maaden 2019**	**Ng 2020 (29 Pts)**				
DNA variant	**c.1388A>G**	**c.320A>G**	**c.320A>G**	**c.320A>G**	**c.320A>G**	**c.320A>G**	**c.320A>G**	**c.320A>G;**	**c.2915G>T;**	**c.241G>A**	**c.50T>A**	**c.207_209delAGA**
Protein var	p.Glu463Gly	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Asn107Ser	p.Gly972Val	p.Ala81Thr	p.Ile17Asn	p.Glu69del
Novel Var	Yes	No	No	No	No	No	No	No	Yes	No	Yes	Yes
Inheritance	Maternal	De novo	De novo	De novo	De novo	ND	De novo	De novo	De novo	De novo	De novo	De novo
Sex	Male	Female	Female	Female	Female	Female	Female	22 Female, 1 male	Male	3 Female	Female	Female
Ancestry	ND	Arabic-UAE	ND	ND	ND	Arabic-UAE	India	ND	ND	ND	ND	ND
Microceph	–	–	Yes	ND	ND	–	Yes	ND	ND	ND	ND	ND
GDD/ID	–	Yes	Yes	Yes	Yes	Yes	Yes	20pts	ND	Yes	Yes	Yes
Seizures	Yes	Yes	Yes	Yes	Yes	Yes	Yes	19 pts	ND	Yes	Yes	Yes

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	Timal 2012	Ligt 2012	Bissar-Tadmouri 2013 (4pts)	Epi4K 2013 Consortium (Allen) (2pts)	Michaud 2014	Hino-Fukuyo 2015	Smith-Packard 2015	Dimasi 2016	Epi4K 2016 Consortium (Myers)	Mooler 2016	Fung 2017	Galama 2017
Brain anomalies	Non-specific WM changes	Cortical atrophy	ND	Cortical atrophy	ND	Cortical atrophy		11 pts: BESS, lack of WM changes, cerebral atrophy, PVL	ND	2 pts: BESS, thinning CC, progressive atrophy	–	–
Hypotonia	Yes	Yes	ND	+	ND	Yes	Yes	22 pts				
Regression	–	–	ND	–	–	–	Yes	ND				
Facial dysmorph	–	Yes	–	ND	ND	Yes	ND	11 pts				
Opthalm. impairment	ND	Yes	–	ND	ND	Yes	ND	12 pts				
Feeding difficulty/GI abnormality	ND	Yes	ND	ND	ND	Yes	ND	11 pts				
Skeletal findings	Yes	ND	–	ND	ND	ND	ND	11 pts				
CDG Testing	TIEF & CDT (MS): normal; Abnormal cellular glycosylation	CDT (HPLC): normal	ND	ND	ND	TIEF: normal	ND	Normal CDT in 14 pts				
Survival	Alive, 3 years	Alive, 26 months	ND	Alive, 3 years	ND	ND	Alive, 30 months	1 pt deceased				
Other	–	–	–	Chorea, dyskinesia	–	Elevated ATIII	–	Cardiac abnormalities in 6 pts; Respiratory abnormalities in 5 pts				

Note: Summary of ALG13-CDG individuals from the literature.

Abbreviations: APTT, activated prothrombin time; BESS, benign enlargement of subarachnoid space; GDD, global developmental delay; G-tube, gastrostomy tube; ID, intellectual disabilities; HPLC, high performance liquid chromatography; ND, not determined; NPCRS, Nijmegan pediatric CDG rating scale; PVL, periventricular leukomalacia; SNHL, sensorineural hearing loss; UAE, United Arab Emirates.