

Further Delineation of the ALG9-CDG Phenotype

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Abstract ALG9-CDG is one of the less frequently reported types of CDG. Here, we summarize the features of six patients with ALG9-CDG reported in the literature and report the features of four additional patients. The patients presented with drug-resistant infantile epilepsy, hypotonia, dysmorphic features, failure to thrive, global developmental disability, and skeletal dysplasia. One patient presented with nonimmune hydrops fetalis. A brain MRI revealed global atrophy with delayed myelination. Exome sequencing identified a novel homozygous mutation c.1075G>A, p.E359K of the ALG9 gene. The results of our analysis of these patients expand the knowledge of ALG9-CDG phenotype.

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Introduction

Congenital disorders of glycosylation (CDG) are genetic disorders caused by defects in the synthesis of the glycans of glycoproteins as well as glycolipids. More than 80 inborn errors of metabolism have been described due to congenital defects in protein N-linked glycosylation. CDG affect multiple organ systems. The severity of symptoms is highly variable and shows a broad clinical spectrum with considerable overlap. An increasing number of disorders have been discovered using new techniques that combine glycobiology with next-generation sequencing (Jaeken 2013; Timal et al. 2012). The majority of CDG known to date represent defects of the dolichol-linked oligosaccharide assembly classified as CDG-I. In 2004, a novel form of CDG, ALG9-CDG, was described caused by a deficiency of alpha-1,2-mannosyltransferase due to a defect in the ALG9 gene (Frank et al. 2004). Here, we describe the clinical, biochemical, and molecular features of four new cases of ALG9-CDG.

Methods

We retrospectively reviewed the clinical and genetic data of the patients. HPLC analysis of serum transferrin isoforms was performed as described (Arndt et al. 2007). The ALG9 mutation was identified using the inborn errors of metabolism gene panel, part of the Mendeliome assay (Saudi Mendeliome Group 2015). Gene panel analysis consisted of removing all non-exonic and non-splice site variants, then utilizing population frequency from our in-house Saudi database to exclude benign polymorphisms. Once the patients were confirmed to have the mutation, the parents were tested. All four patients had a skeletal survey, a brain

MRI, US of the abdomen and kidneys, an echocardiogram, and an EEG.

Results

We have evaluated four patients from a large consanguineous family (pedigree). The main clinical features are summarized in Table 1.

The Mendeliome assay revealed a previously reported homozygous missense mutation (NM_024740.2: c.1588G>A:p.E530K) in the *ALG9* gene. Sanger sequencing confirmed this mutation. The parents were tested and found to be heterozygous for the mutation.

The index case (IV:5) is a 6-year-old female. The mother mentioned decreased fetal movements during pregnancy. The patient was the product of a full-term normal vertex delivery with a birth weight of 3,170 g, length of 49 cm, and head circumference of 34 cm. Apgar score was 8 at 1 min and 9 at 5 min. She was diagnosed since birth to have hip dislocation, dysmorphic features, and congenital heart disease in the form of minor tricuspid regurgitation.

At the age of 4 months, the patient started to develop frequent seizure attacks in the form of frequent clusters of clonic and tonic movements with up-rolling of the eyes. She was frequently admitted for uncontrolled seizures and unexplained febrile episodes. The EEG showed bursts of spikes/sharp waves in the left parieto-central area with slow background activity in favor of epileptic encephalopathy. After the first 5 years of life, the patient became seizure-free with a normal EEG.

The patient showed global developmental disability. At the age of 6 years, she can sit without support, reach for objects, make incomprehensible sounds, turn her head toward a voice, and recognize her family.

On examination at the age of 6 years, all growth parameters were at the 5th percentile. She was fixing but not following. She was hypotonic and showed exaggerated deep tendon reflexes but without clonus.

Facial dysmorphism includes frontal bossing, depressed nasal bridge, low seated ears, large mouth, and hypertelorism. Other associated abnormalities were inverted widely spaced nipples, abnormal distribution of fat on the buttocks, cutis marmorata, cutis aplasia congenita, and broad thumbs (Fig. 1). No organomegaly was noted.

HPLC analysis of the transferrin isoforms showed a type 1 pattern (elevated diasialotransferrin (28%; reference value: 1.10 ± 0.72) and asialotransferrin (2%; reference value: not detectable). The activity of phosphomannomutase and lipid-linked oligosaccharides in fibroblasts were normal. The skeletal survey showed delayed bone age and

mild skeletal dysplasia including mesomelic brachymelia with thickening of frontal and occipital bone, mild kyphosis of thoracolumbar spine, bilateral hip dislocation, round pelvis, brachycephaly, and shortening of greater sciatic notch. There are 11 ribs, symmetric with normal shape. The electroretinogram was normal. Brain MRI showed global cerebrum and cerebellar atrophy with delayed myelination.

The family history revealed three affected cousins (Fig. 2). Patients IV:8 and IV:13 have a similar presentation as patient IV:5 (Table 1). The only difference is that patient IV:8 has less severe developmental disability.

Patient IV:3 is a 25-day-old boy. He was diagnosed by fetal ultrasonography at 28 weeks of gestation with hydrops fetalis, which consisted of severe skin edema, pericardial effusion, and ascites. At 37 weeks of gestation, a cesarean section was performed because of breech presentation. Birth weight was 3,700 g (above 97th centile). Apgar scores were 7 at 1 min and 9 at 5 min. He had neck, feet, and skin edema. He had facial dysmorphism including a short nose, with a long philtrum, and a short neck. Echocardiography showed an atrial septal defect and mild dilatation of the right ventricle. Abdominal ultrasound was normal. Skeletal survey showed mild skeletal dysplasia. Cranial ultrasound showed wide subarachnoid spaces.

Discussion

So far only six patients with an *ALG9*-related phenotype have been reported in the literature (Frank et al. 2004; Tham et al. 2015; Vleugels et al. 2009; Weinstein et al. 2005). In this paper we report four more patients. Our patients' phenotype, except patient IV:3, is similar to that of the patients described by Frank et al. (2004), Vleugels et al. (2009), and Weinstein et al. (2005). All of these patients, including our three, presented with a phenotype including infantile epileptic encephalopathy, progressive microcephaly, failure to thrive, and global developmental disability. Hepatomegaly, cardiac anomalies, mild skeletal dysplasia, and dysmorphism were observed in some patients. Hydrops fetalis was observed in only one patient. The three patients reported by Tham et al. had more severe phenotype (severe skeletal dysplasia, polycystic kidney, and multiple malformations) similar to that first reported by Gillissen-Kaesbach and Nishimura (Gillissen-Kaesbach et al. 1993; Nishimura et al. 1998). All of these patients died in utero. It is possible that the milder phenotypes of patients who were reported previously may be explained by a residual activity of the defective enzymes.

Our study confirms that skeletal dysplasia is a common symptom in *ALG9*-CDG syndrome, but this feature is also

Table 1 Clinical and molecular characteristics of reported and present patients with ALG9-CDG

	Patient (Frank et al. 2004)	Patient (Weinstein et al. 2005)	Patient (Vleugels et al. 2009)	Patients (3 patients) (Tham et al. 2015)	Patient 1	Patient 2	Patient 3	Patient 4 (25 days old)
Epilepsy	+	+	+		+	+	+	-
Developmental disability	+	+	+		+	+	+	-
Microcephaly	+	+	?		+	+	+	-
Dysmorphism	NA	+	+	+	+	+	+	+
Skeletal dysplasia	NA	NA	NA	Severe	Mild	Mild	Mild	+
Hepatomegaly	+	+	+		Mild	Mild	Mild	Mild
Failure to thrive	NA	+	+		+	+	+	-
Hydrops fetalis	-	-	-	-	-	-	-	+
Echocardiography	NA	Pericardial effusion	Pericardial effusion	CHD (1 patient): large ventricular septum defect and a double outlet right ventricle with anomalous outflow tracts	Mild tricuspid regurgitation	Normal	Mild pericardial effusion	Atrial septal defect, mild dilatation of right ventricle
US kidneys	NA	Polycystic kidneys	NA	No CHD in 2 patients Polycystic kidneys	Normal	Normal	Normal	Normal
Brain MRI	NA	Cerebral and cerebellar atrophy, delayed myelination	Normal (CT)		Cerebral and cerebellar atrophy, delayed myelination	Cerebral and cerebellar atrophy, delayed myelination	Cerebral and cerebellar atrophy, delayed myelination	US head normal
ALG9 mutation	c.1567G>A (p.E523K)	c.860A>G (p.Y286C)	c.860A>G (p.Y286C)	c.1173+2T>A (p.V340A/s*57)	c.1588G>A (p.E530K)	c.1588G>A (p.E530K)	c.1588G>A (p.E530K)	c.1588G>A (p.E530K)

NA information not available, CHD congenital heart disease
Please note that in Saudi Mendeliome Group (2015), there are three patients

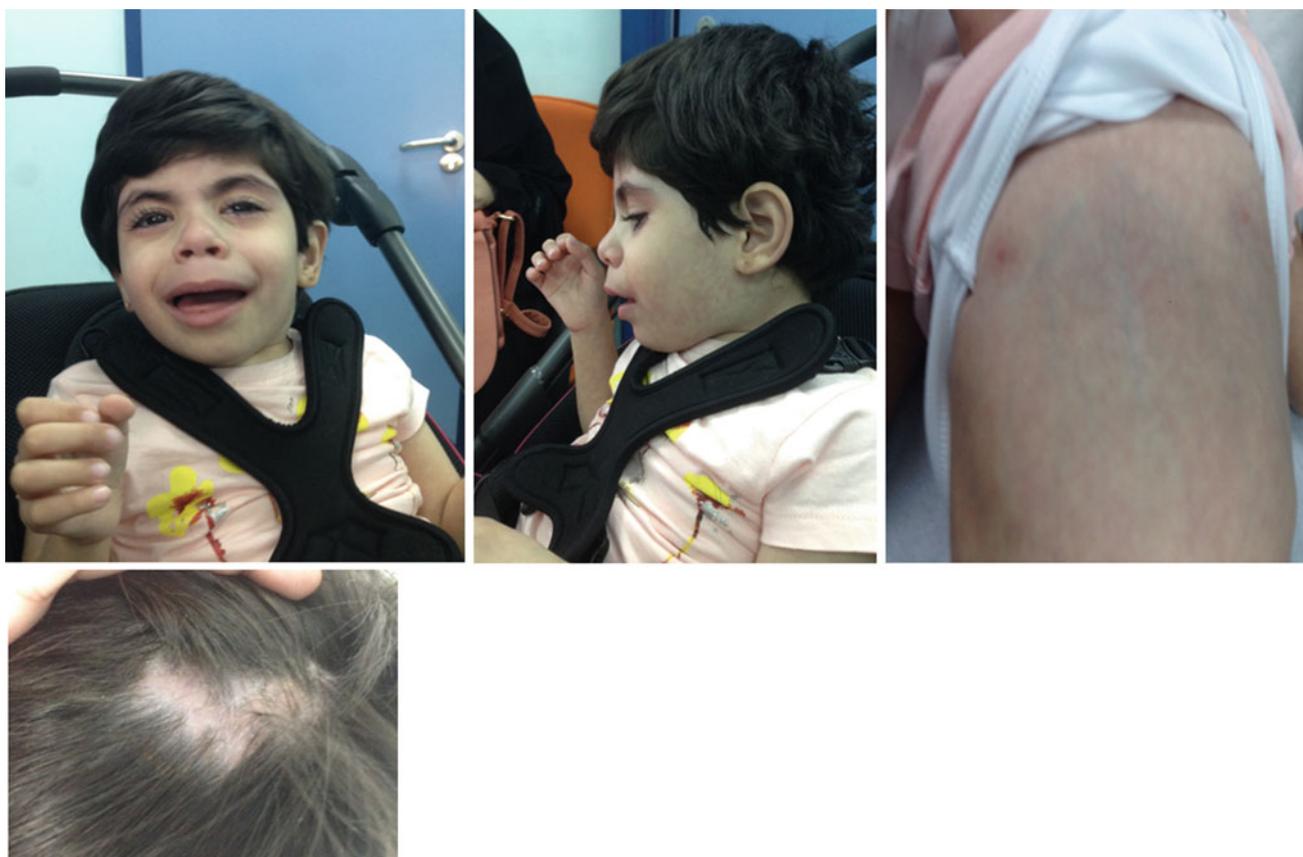


Fig. 1 Photographs of the index case showing craniofacial dysmorphism (partial aplasia cutis congenita of the scalp, hypotelorism, broad base of nose, upturned nose, big mouth, prominent maxilla,

large ears), inverted widely spaced nipples, abnormal distribution of fat at the buttocks, cutis marmorata, and broad thumbs

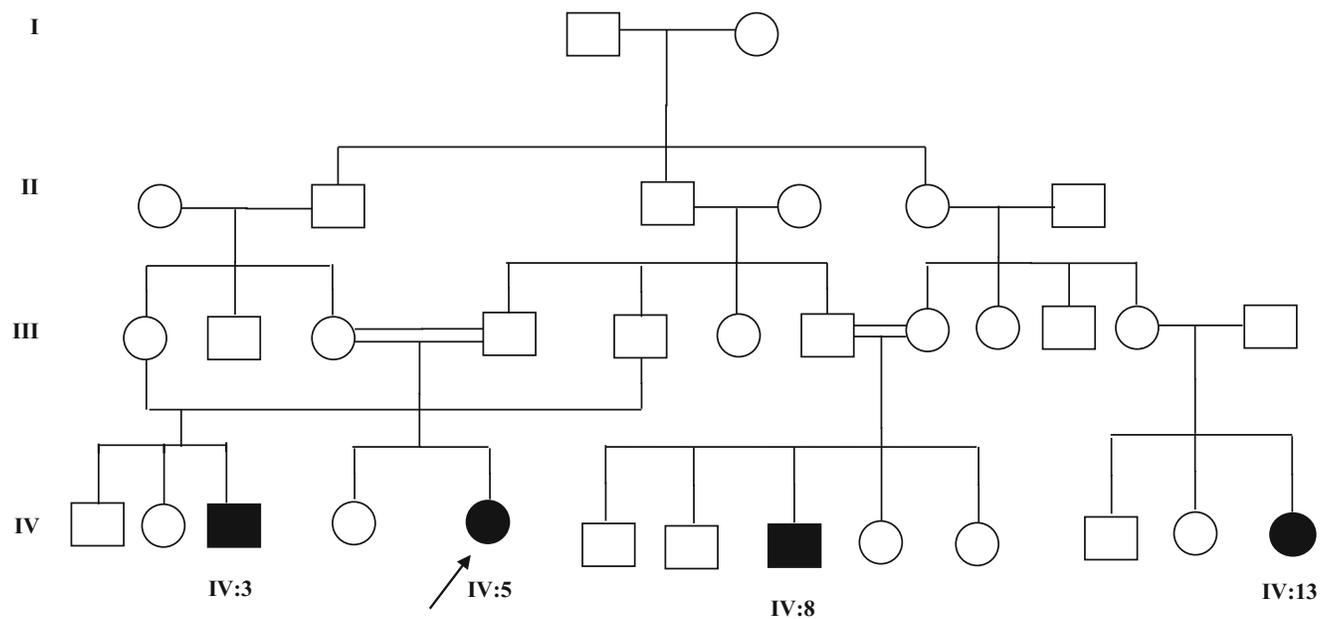


Fig. 2 Pedigree of the family. The index case is indicated by an *arrow*

observed in other CDG including ALG3-CDG and ALG12-CDG (Lepais et al. 2015; Murali et al. 2014). Our study reports for the first time hydrops fetalis in ALG9-CDG. Also patients with PMM2-CDG and ALG8-CDG may present with hydrops fetalis (Höck et al. 2015; van de Kamp et al. 2007).

Although the number of patients is still small, there seems to be some phenotype-genotype correlation (Table 1). Patients with the most severe phenotype exhibit severe lethal skeletal dysplasia with visceral malformations. Their genotype is homozygous for a deleterious variant in the splice donor site of exon 10 in ALG9 (NG_009210.1:g. also denoted NM_024740.2:c.1173+2T>A). This variant results in skipping of exon 10 and leads to a shorter transcript. The other patients exhibit epilepsy and microcephaly, developmental disability, hepatomegaly, mild skeletal dysplasia, and cardiac anomalies. Interestingly, after an aggressive phase after 4–5 years with drug-resistant epilepsy and severe hypotonia, these patients become more stable and show some improvement in their milestones and epilepsy. Their genotype is a homozygous missense mutation.

In conclusion, the clinical and the biochemical findings described in these patients add to the spectrum of presentations of ALG9-CDG. Skeletal dysplasia, ranging in severity from mild to severe, appears to be a consistent feature. Other clinical features common to other forms of CDG are often associated including nonimmune hydrops fetalis.

Synopsis

The ALG9-CDG phenotype commonly includes skeletal dysplasia and may present with hydrops fetalis. A review of the literature suggests genotype-phenotype correlation.

Compliance with the Ethics Guidelines

Conflict of Interest

Sarah AlSubhi, Amal Hashem, Kalthoum Tlili, Saad AlShahwan, Dirk Lefeber, Fowzan S. Alkuraya, and Brahim Tabarki declare that they have no conflict of interest.

Details of the Contributions of Individual Authors

All authors (Sarah AlSubhi, Amal Hashem, Kalthoum Tlili, Saad AlShahwan, Dirk Lefeber, Fowzan S. Alkuraya, Brahim Tabarki) contributed equally in the planning, conducting, and reporting of the work described in the article.

Name of one author who serves as guarantor for the article, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish: Brahim Tabarki

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Details of Ethics Approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki.

In the declaration of 1975, as revised in 2000, informed consent was obtained from the family for being included in the study.

A Patient Consent Statement

A consent form for the figures has been signed by the parents.

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