Defining the Phenotype in Congenital Disorder of Glycosylation Due to ALG1 Mutations

abstract

Deficiency of β-1,4 mannosyltransferase (MT-1) congenital disorder of glycosylation (CDG), due to ALG1 gene mutations. Features in 9 patients reported previously consisted of prenatal growth retardation, pregnancy-induced maternal hypertension and fetal hydrops. Four patients died before 5 years of age, and survivors showed a severe psychomotor retardation. We report on 7 patients with psychomotor delay, microcephaly, strabismus and coagulation abnormalities, seizures and abnormal fat distribution. Four children had a stable clinical course, two had visual impairment, and 1 had hearing loss. Thrombotic and vascular events led to deterioration of the clinical outcome in 2 patients. Four novel ALG1 mutations were identified. Pathogenicity was determined in alg1 yeast mutants transformed with hALG1. Functional analyses showed all novel mutations representing hypomorphs associated with residual enzyme activity. We extend the phenotypic spectrum including the first description of deafness in MT1 deficiency, and report on mildly affected patients, surviving to adulthood. The dysmorphic features, including abnormal fat distribution and strabismus highly resemble CDG due to phosphomannomutase-2 deficiency (PMM2-CDG), the most common type of CDG. We suggest testing for ALG1 mutations in unsolved CDG patients with a type 1 transferrin isoelectric focusing pattern, especially with epilepsy, severe visual loss and hemorrhagic/thrombotic events. Pediatrics 2012;130:e1034–e1039

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KEY WORDS
β-1,4 mannosyltransferase, CDG-Ik, short chain lipid-linked oligosaccharides, seizures, microcephaly

ABBREVIATIONS
ALG—asparagine-linked glycosylation
CDG—congenital disorders of glycosylation
CPY—carboxypeptidase Y
GDP—mannose—GlcNAc2-PP-dolichol β1,4-mannosyltransferase
LLO—lipid-linked oligosaccharides
MT-1—β-1,4 mannosyltransferase
PMM—phosphomannomutase
TIEF—transferrin isoelectric focusing

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1Drs Bodamer, Lehle, and Wevers are last authors with equal contribution.

All authors have participated in the concept and design, analysis and interpretation of data, drafting or revising of the manuscript, and all authors have approved the manuscript as submitted.

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INTRODUCTION

Congenital disorders of glycosylation (CDG) represent a clinically diverse group of inborn errors, with a rapidly increasing number of patients.1–5 Protein glycosylation is important in post-translational modification and has an effect on the majority of functional proteins.6–8 Glycosylation disorders affect N-linked and O-linked glycosylation, combined N- and O-linked glycosylation, and dolichol synthesis.7 Screening for glycosylation defects by serum transferrin isoelectric focusing (TIEF) or capillary zone electrophoresis enables discrimination between defects of lipid-linked oligosaccharide (LLO) assembly and oligosaccharide transfer to protein, defined as CDG-I, and defects of N-linked glycan processing grouped as CDG-II.9 The definitive diagnosis in CDG relies on enzyme measurements and genetic testing.

The most common N-glycan biosynthesis defect is PMM2-CDG (previously CDG Ia) with almost 700 reported patients.2 This recognizable multisystem disease shows discriminative features, like fat pads, inverted nipples and endocrine and coagulation abnormalities. A newly emerging, apparently underdiagnosed group of disorders involving the early steps of the N-glycan biosynthesis (SRD5A3-CDG, DK1-CDG, ALG1-CDG) have also a recognizable clinical presentation with severe visual loss, cerebellar involvement, and encephalopathy.

Screening for glycosylation defects is relatively easy. Separation of serum transferrin isoforms by isoelectric focusing (TIEF), capillary zone electrophoresis or HPLC enables the detection of N-linked glycosylation defects. The tests allow discrimination between defects of lipid-linked oligosaccharide assembly and oligosaccharide transfer to protein, defined as CDG-I, and defects of N-linked glycan processing grouped as CDG-II.9 The definitive diagnosis in CDG relies on enzyme measurements and genetic testing.

The CDG subtype asparagine-linked glycosylation (ALG)1-CDG (MIM #608540, CDG-Ik according to previous nomenclature) is caused by a deficiency of β1,4-mannosyltransferase (MT-1, MIM*605907). Previously, 9 pediatric patients have been reported to have ALG1-CDG.10–13 Three ALG1-CDG patients had a lethal
form with severe multiorgan involvement, generalized edema, epilepsy, coagulation abnormalities, and death in the first months of life. One patient died of respiratory insufficiency at age 4 years. So far all reported patients demonstrated a profound neurologic involvement with hypotonia, seizures, developmental delay, and visual disturbances. Here we report on 7 additional ALG1-CDG patients carrying novel mutations including the first description of deafness and mildly affected patients, surviving to adulthood.

CASE REPORT

Patients

We investigated 3 separate families with 7 patients (4 female and 3 male patients) diagnosed with CDG-Ix. Two patients were of European ancestry (Austrian and Polish ancestry) and 5 individuals of Turkish ancestry (Fig 1). Patient IV/13 was described without the biochemical/genetic diagnosis previously. Serum TIEF revealed a type 1 profile characterized by significant increase of disialo- and asialotransferrin and low tetrasialotransferrin in the probands from three families (IV/14, II/2, and II/3; see Supplementary Table 2). Secondary glycosylation defects and PMM2-CDG and phosphomannose isomerase-CDG were excluded by enzyme activity measurements in cultured fibroblasts (Figs 2 and 3). LLO analysis after [3H] mannose labeling was normal in these patients (see Supplementary Information).

Family 1

Patient IV/13 had hypotonia and strabismus, microcephaly, and mild psychomotor delay. At age 21 years, she shows mild intellectual disability, but no seizures or spontaneous hemorrhages. Patient IV/14 deceased at age 10 years after surgery for Budd-Chiari syndrome. Patient IV/18 had severe congenital hypotonia, ophthalmoplegia, ataxia, intractable seizures, peripheral neuropathy, and recurrent bacterial infections. Patient V/2 had microcephaly, hypotonia, strabismus, intractable seizures, ataxia, recurrent bacterial infections, and moderate developmental delay. Patient V/5 presented with dysmorphic features and strabismus, axial hypotonia, failure to thrive, recurrent gastrointestinal infections, complicated febrile seizures, spontaneous hemorrhages, microcephaly, ataxia, and developmental delay.

Family 2

Patient II/2 developed deafness and visual loss, and, after an infection associated with nephrotic syndrome and a venous thrombosis, he died at 5 months.

FIGURE 3

A, Glycosylation status of carboxypeptidase Y (CPY). Complementation of the glycosylation defect of the temperature-sensitive alg1 yeast mutant by hALG1. The glycosylation status of CPY is shown at the permissive temperature (25°C, left panel) and at the nonpermissive temperature (36°C, right panel). Yeast cells transformed with hALG1 from a control or alg1 patients (hALG1S359L and hALG1S258L, respectively) were labeled with [35S]-methionine for 30 minutes, and CPY was immunoprecipitated and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The positions of the mature form of CPY (mCPY, in wild-type yeast cells (lane 1) and underglycosylated forms in alg1 cells (lanes 2–7) are indicated. Molecular weight standards are shown on the left. B, Growth of yeast alg1 cells either transformed with wild-type hALG1, hALG1S359L, hALG1S258L, or the empty expression vector. Growth was investigated under permissive (25°C) or nonpermissive (36°C) growth temperature.
<table>
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<th>Fam. 1 IV/13</th>
<th>Fam. 1 IV/14</th>
<th>Fam. 1 IV/18</th>
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<th>Fam. 1 IV/5</th>
<th>Fam. 2 II/2</th>
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<td>Gender F M</td>
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<td>10 y 9 mo</td>
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<td>10 y 1 mo</td>
<td>4 y – 9 y</td>
<td>4 y – 20 y 8 mo</td>
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<td>Age at death –</td>
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<td>7m</td>
<td>8m</td>
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<td>5m</td>
<td>6/7</td>
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<td>1y9m</td>
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<td>8m</td>
<td>0/5</td>
<td>5/12</td>
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<td>Psychomotor delay –</td>
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<td>Splenic involvement –</td>
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<tr>
<td>Severe Infections or Episodes of Unexplained Fever –</td>
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<td>+</td>
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<td>ALG1 mutation c.1076C&gt;T/c.1076C&gt;T</td>
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F, female; M, male; NR, not recorded; –, not present; +, mild; ++, moderate; ++++, severe.
Family 3

Patient (II/3) presented with microcephaly, dysmorphic features, inverted nipples, strabismus, axial hypotonia, and feeding difficulties. The visual maturation was delayed; she developed therapy-resistant epilepsy and had a cerebral infarct, spontaneous hemorrhages, and thrombosis. Currently at age 10 years, he has severe psychomotor retardation, decreased visual acuity, epilepsy, ataxia and is wheelchair bound.

The clinical findings and genotypes are compiled in Table 1. All patients had neurologic and visual involvement, PMR, ataxia, dysmorphic features (large ears, fat pads and inverted nipples), abnormal coagulation, and a happy character; similar to PMM2-CDG patients. The clinical features and the genetic findings of all known 16 ALG1-CDG patients are summarized in Table 1 and Supplemental Table 3. (For linkage analysis, mutations, conservation, and structural analyses of mutations, MT-1 enzyme assay and functional analysis of the mutations in yeast see the Supplemental Information and Supplemental Figs 4 and 5.)

DISCUSSION

According to the EUROGLYCANET database, ALG1-CDG currently represents the fourth most common CDG-I form in Europe, after PMM2-CDG, ALG6-CDG, and SRD5A3-CDG. Conventional LLO analysis by labeling cells with (2-3H)-mannose shows normal results in ALG1-CDG fibroblasts. The technique lacks sensitivity for detection of short-LLO synthesis defects. It is therefore likely that there are more ALG1-CDG patients who have been missed and defined as unsolved CDG (CDG-bx) after conventional LLO analysis.

Initially, ALG1-CDG was diagnosed with a severe phenotype compared with other CDG types, with prenatal hydrops, hypotonia, intractable seizures, and death in the first year of life. Recently, 5 pediatric patients were reported with severe visual disturbances, profound developmental delay, and one early death (Supplemental Table 3). In contrast, 6 of 7 cases from our cohort initially presented with a more benign form of ALG1-CDG, stable clinical course, and survival into infancy and adulthood, demonstrating that ALG1-CDG comprises a wide phenotypic spectrum. In 2 of these 6 patients, coagulation events led to sudden deterioration, suggesting disease variability in ALG1-CDG correlating with vascular events. Interestingly, an ALG6 S304F polymorphism was reported to be a genetic modifier for the clinical outcome in PMM2-CDG. However, this was tested and excluded as an explanation in our ALG1-CDG cohort (all patients were homozygous for F304).

Here we define a recognizable phenotype in ALG1-CDG. On the basis of a total of 16 patients, the ALG1-CDG phenotype comprises of microcephaly, developmental delay, abnormal fat distribution, strabismus, and altered blood coagulation with a high probability of hemorrhages or thromboses. These events represent major causes for mortality and morbidity. Eye involvement (strabismus, nystagmus, and in some patients severe visual loss), ataxia, and seizures are present in most patients. (Intractable seizures occurred in 10 of 14 patients with onset generally within the first year of life in our cohort.)

Most of the clinical features overlap with those of PMM2-CDG, the most common subtype of CDG. One should note that compared with PMM2-CDG severe visual disturbance and microcephaly are more common but vermis hypoplasia is seldom detected in ALG1-CDG patients. A growing group of clinically recognizable disorders of the early steps of the N-glycan biosynthesis (SRD5A3-CDG, DK1-CDG, and ALG1-CDG) have also overlapping clinical features, including visual loss, ataxia, and encephalopathy. Those patients, however, have no abnormal fat pads and DK1-CDG shows a different LLO pattern by fibroblast analysis. Compared with other types of CDG, ALG1-CDG is also unique because of a high incidence of manifest bleeding abnormalities and thrombotic events in patients.

With the identification of sensorineural hearing loss in 1 of our patients, we also extended the phenotypic spectrum with the first description of deafness in ALG1-CDG, which is in general a unique feature in CDG. The description of milder cases and data on the case followed up until age 21 years demonstrate that ALG1-CDG patients can survive to adulthood. ALG1-CDG could have been missed in the past because the diagnosis has not been anticipated in patients presenting with mild or moderate phenotype. The current report underpins that CDG should be suspected and screened by transferrin analysis in any child with a multisystem disease, especially in combination with neurologic symptoms and abnormal coagulation parameters.

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REFERENCES

1. Jaeken J, Vanderschueren-Lodeweyckx M, Casaer P. Familial psychomotor retardation with markedly fluctuating serum proteins, FSH and GH levels, partial TBG-deficiency, increased serum arylsulfatase A and increased CSF protein;
7. Jaeken J, Hennet T, Matthijs G, Freeze HH. CDG nomenclature: time for a change! Biochim Biophys Acta 2009;1792:825–826.

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