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Brief Communication

Severe, fatal multisystem manifestations in a patient with dolichol kinase-congenital disorder of glycosylation

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ABSTRACT

Congenital disorders of glycosylation are a group of metabolic disorders with an expansive and highly variable clinical presentation caused by abnormal glycosylation of proteins and lipids. Dolichol kinase (DOLK) catalyzes the final step in biosynthesis of dolichol phosphate (Dol-P), which is the oligosaccharide carrier required for protein N-glycosylation. Human DOLK deficiency, also known as DOLK-CDG or CDG-Im, results in a syndrome that has been reported to manifest with dilated cardiomyopathy of variable severity. A male neonate born to non-consanguineous parents of Palestinian origin presented with dysmorphic features, genital abnormalities, talipes equinovarus, and severe, refractory generalized seizures. Additional multi-systemic manifestations developed including dilated cardiomyopathy, hepatomegaly, severe insulin-resistant hyperglycemia, and renal failure, which were ultimately fatal at age 9 months. Electrospray ionization mass spectrometric (ESI-MS) analysis of transferrin identified a type I congenital disorder of glycosylation; next-generation sequencing demonstrated homozygous p.Q483K *DOLK* mutations that were confirmed in patient fibroblasts to result in severely reduced substrate binding and catalytic activity. This patient expands the phenotype of DOLK-CDG to include anatomic malformations and multi-systemic dysfunction.

1. Introduction

Congenital disorders of glycosylation (CDGs) are a group of metabolic disorders caused by abnormal protein and lipid glycosylation, a co- and post-translational addition of carbohydrate moieties essential for protein folding, stability, and cell–cell adhesion [1]. N-linked glycosylation involves transfer of pre-assembled oligosaccharides from dolichol-pyrophosphate to an asparagine residue of a protein in the endoplasmic reticulum [1]. Isoelectric focusing (IEF), electrospray ionization mass spectrometry (ESI-MS), high performance liquid chromatography (HPLC), and capillary electrophoresis (CE) are used to search for patterns of underglycosylated serum transferrin seen in N-linked CDGs [2]. Because glycosylation is a fundamental process essential to protein and lipid function, CDGs have pleiotropic effects that often have severe or fatal manifestations in affected individuals. Symptoms generally associated with CDGs include profound global delay, epilepsy, polyneuropathy, ataxia, endocrine abnormalities, ichthyosis, visual and hearing loss, cardiac, liver, renal, and gastrointestinal involvement [3].

In 2007, a CDG involving a defect in dolichol kinase [DOLK, E.C. 2.7.1.108], the enzyme catalyzing the final step in the biosynthesis of dolichol phosphate (Dol-P), was discovered [4]. Dolichol phosphate is essential for N-glycosylation due to its role as the oligosaccharide carrier upon which the lipid-linked oligosaccharide (LLO) precursor is assembled [5]. Dol-P, in the form of Dol-P-Mannose, also acts as a mannose donor for LLO synthesis, O-, C-mannosylation, and glycerol phosphatidylinositol anchor synthesis [5]. The clinical spectrum of previously reported patients with DOLK-CDG (refer to Table 1 for a summary of their symptoms and *DOLK* mutations), also known as CDG-Im (OMIM # 610768), includes seizure disorder and developmental delay [6], progressive dilated cardiomyopathy, severe hypotonia, and ichthyosis [5]. We report a patient with DOLK-CDG sharing symptoms with those patients, but also presenting novel clinical manifestations that expand the DOLK-CDG phenotype.

Abbreviations: CTP, cytosine triphosphate; EDTA, ethylenediaminetetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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2. Case report

The male patient was born following an uncomplicated pregnancy except for an instance of first trimester bleeding, to a 23-year-old primigravida and a 26-year-old father of nonconsanguineous Palestinian origin. He was delivered full-term by cesarean due to non-reassuring fetal heart rate and meconium-stained amniotic fluid. Physical examination showed systolic heart murmur, hypotonia, bilateral talipes equinovarus, sacral dimple with hairy tuft, localized lower extremity hypertrichosis, and penoscrotal fusion. No other skin abnormalities or optic atrophy was noted. Echocardiogram showed no evidence of cardiac dilatation or abnormal function. He had good initial respiratory effort; however, shortly thereafter manifested repeated episodes of apnea, cyanosis, and bradycardia. The events were found to be caused by partial or generalized seizures, as an electroencephalogram revealed non-specific encephalopathy and generalized multifocal seizures. The apneic episodes occurred so frequently that he was intubated and mechanically ventilated. Brain imaging demonstrated diffuse cortical atrophy and delayed myelination, particularly in the cerebellar hemispheres. He had gastroesophageal reflux and dysphagia, failed to thrive, and eventually required a gastrostomy tube for enteral feeding. N-linked CDG was suspected when ESI-MS of transferrin (Mayo Clinic, Rochester, MN) demonstrated elevated *mono-oligosaccharide:di-oligosaccharide transferrin* ratio of 0.707 (reference range < 0.100) and *a-oligosaccharide:di-oligosaccharide transferrin* ratio of 0.216 (reference range < 0.050). These ratios are indicative of Type I CDG, resulting in impaired synthesis or transfer of the LLO precursor that subsequently generates proteins with unoccupied glycosylation sites [2]. This is in comparison to Type II CDGs, which are caused by impaired processing, such as trimming and remodeling, of the protein-bound oligosaccharide, creating proteins that have fully occupied glycosylation sites but with abnormal glycans [2].

At age 3 months, the patient developed sinus tachycardia, hypertension, unexplained hypokalemia (K^+ 3.0 mEq/L, reference range 3.6–6.0 mEq/L), and hyperglycemia (glucose 461 mg/dL, reference range 65–110 mg/dL) despite low glucose infusion rate of 2 mg/kg/min. While potassium levels eventually stabilized, his glucose remained markedly elevated without ketoacidosis. Even high continuous insulin infusion (1.3 units/kg/h) did not stabilize the glucose. He developed hepatomegaly with elevated aspartate aminotransferase (174 units/L, reference range 22–58 units/L), alanine aminotransferase (121 units/L, reference range 11–39 units/L), and alkaline phosphatase (1310 units/L, reference range 100–302 units/L). Prothrombin international normalized ratio was 1.1 (reference range 0.8–1.2 s) and partial thromboplastin time was 30.3 (reference range 23–40 s). Creatine phosphokinase was not elevated at 66 (reference range 41–277 units/L). Clinical deterioration was evidenced by absent response to stimulation or primitive reflexes, sluggish pupils, hypotonia, and bilateral ankle clonus.

During his last four days of life, he developed wide-complex tachycardia with atrioventricular dissociation, and his cardiac function rapidly deteriorated with a decline in contractile function (20.3%, reference range > 28%) and ejection fraction (43.6%, reference range > 55%). Borderline cardiac dilatation, concentric left ventricular hypertrophy, and decreased left ventricular function were noted. He developed renal failure and marked abdominal distension secondary to hepatomegaly and ascites (albumin 2.5 g/dL, reference range 2.7–4.8 g/dL). He was noted to have dysconjugate gaze, sluggishly reactive pupillary light reflex, and became non-responsive and hypotonic with occasional spontaneous four-extremity clonus. He expired at 4 months from combined cardiac, renal, and liver failure.

3. Methods

3.1. Human subjects

The study was granted exempt status by the institutional review board of Children's Hospital of Orange County as IRB study #130657.

3.2. Identification of DOLK mutations

Patient DNA was analyzed using a targeted 47 gene next generation sequencing panel specific for disorders of glycosylation [7]. This panel utilized RainDance Technologies™ microdroplet enrichment of the targeted region, which included all exons for each of the 47 genes and at least 25 nucleotides upstream and downstream of each exon. After enrichment, samples were run on a SOLiD™ 3 Plus system (LifeTechnologies, Carlsbad, CA), and the average coverage for this sample was 1200× with 87% of the nucleotides having 100× coverage or greater. Bioinformatic filtering for the known SNPs assisted with the identification of changes that were most likely novel and therefore warranted further consideration and confirmation by Sanger sequencing.

3.3. Preparation of microsomal fractions from cultured human cells

Human cells from primary cultures were incubated in PBS containing 10 mM EDTA, collected by centrifugation (1000 ×*g*, 10 min), resuspended in ice-cold 10 mM HEPES-NaOH, pH 7.4, 0.25 M sucrose, 1 mM dithiothreitol, and lysed by probe sonication with a Kontes Micro Ultrasonic Cell Disruptor (40% full power, 15 s, 2 pulses). Homogenates were sedimented at 1000 ×*g*, 10 min, to remove unbroken cells, and microsomes were recovered from the supernatant by centrifugation at 100,000 ×*g*, 20 min in a Beckman TL-100 Ultracentrifuge. Microsomes were washed with lysis buffer one time, resuspended to ~10 mg/mL protein in lysis buffer and stored at −20 °C until analysis.

3.4. In vitro analysis of dolichol kinase activity

Reaction mixtures for dolichol kinase activity contained 50 millimolar (mM) Tris-Cl (pH 8.0), 10 mM $CaCl_2$, 5 mM NaF, 1 mM sodium orthovanadate, 5 mM dithiothreitol, 10 mM UTP, 0.025% Nonidet P-40, 100 micromolar (μM) dolichol (dispersed in 0.5% Nonidet P-40 by probe sonication), microsomal fraction from cultured CDG cells (50 μg of membrane protein, prepared as described previously), 0.125 M sucrose and the indicated concentration of [γ-^{32}P]CTP (5 to 40 μM, 745 counts per minute/pmol) in a total volume of 0.01 mL. Following incubation at 37 °C for 10 min, the enzymatic products were extracted into $CHCl_3/CH_3OH$ and assayed as described previously [8].

4. Results

Next-generation sequencing identified homozygous c.1447C>A (p.Q483K) *DOLK* mutations; both parents were heterozygous carriers. Functional studies showed that the mutant DOLK enzyme demonstrated reduced V_{max} (100 pmol/min, controls 238 and 417 pmol/min) and increased K_m (36 μM, controls 10.2 and 10.4 μM), confirming that the *DOLK* p.Q483K mutation encodes a form of dolichol kinase with severely deficient enzymatic activity (Fig. 1).

5. Discussion

Our case adds to the existing clinical phenotype of DOLK-CDG patients with a different spectrum of multisystemic involvement compared to other cases. Previously reported DOLK-CDG patients who died suffered mainly from dilated cardiomyopathy [5] or seizure disorder [6]. While our patient developed heart failure and seizures, he also had multiple major malformations such as talipes equinovarus and penoscrotal fusion, which have not yet been reported in DOLK-CDG patients. In addition, he had severe insulin-resistant hyperglycemia, which is different from the hyperinsulinemic hypoglycemia typically seen in the type I CDGs [2]. He also developed renal failure and hepatic synthetic dysfunction as evidenced by electrolyte disturbances, anuria, hepatomegaly, elevated transaminases, and ascites.