

Mitochondrial Complex III Deficiency with Ketoacidosis and Hyperglycemia Mimicking Neonatal Diabetes

Nataschia Anastasio • Maja Tarailo-Graovac •
Reem Al-Khalifah • Laurent Legault •
Britt Drogemoller • Colin J.D. Ross •
Wyeth W. Wasserman • Clara van Karnebeek •
Daniela Buhas

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Abstract Hyperglycemia is a rare presenting symptom of mitochondrial disorders. We report a case of a young girl who presented shortly after birth with ketoacidosis, hyperlactatemia, hyperammonemia, and insulin-responsive hyperglycemia. Initial metabolic work-up suggested mitochondrial dysfunction. Given our patient's unusual presen-

tation, whole-exome sequencing (WES) was performed on the parent-offspring trio. The patient was homozygous for the c.643C>T (p.Leu215Phe) variant in *CYCI*, a nuclear gene which encodes cytochrome *c*₁, a subunit of respiratory chain complex III. Variants in this gene have only been previously reported in two patients with similar presentation, one of whom carries the same variant as our patient who is also of Sri Lankan origin.

Primary complex III deficiencies are rare and its phenotypes can vary significantly, even among patients with the same genotype.

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N. Anastasio (✉) • D. Buhas
Department of Medical Genetics, McGill University, 1001 Boulevard Décarie, Montréal, QC, Canada H4A 3J1
e-mail: natascia.anastasio@mail.mcgill.ca

M. Tarailo-Graovac
Centre for Molecular Medicine and Therapeutics, University of British Columbia, 950 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4

R. Al-Khalifah • L. Legault
Division of Pediatrics Endocrinology, McGill University, 1001 Boulevard Décarie, Montréal, QC, Canada H4A 3J1

R. Al-Khalifah
Division of Pediatric Endocrinology, King Saud University, Riyadh, Saudi Arabia

B. Drogemoller
Child & Family Research Institute, University of British Columbia, 938 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4

C.J.D. Ross
Child & Family Research Institute, University of British Columbia, 950 West 28th Avenue, A3-216, Vancouver, BC, Canada V5Z 4H4

W.W. Wasserman
Department of Medical Genetics, Centre for Molecular Medicine and Therapeutics, Child & Family Research Institute, University of British Columbia, 950 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4

C. van Karnebeek
Department of Pediatrics, Centre for Molecular Medicine and Therapeutics, Child & Family Research Institute, University of British Columbia, 950 West 28th Avenue, Vancouver, BC, Canada V5Z 4H4

Introduction

Complex III (CIII) forms the central part of the mitochondrial respiratory chain. CIII oxidizes coenzyme Q, reduces cytochrome *c*, and pumps protons from the mitochondrial matrix to the inter-membrane space through the Q-cycle mechanism. CIII is composed of 11 subunits. Cytochrome *c*₁, cytochrome *b*, and the Rieske protein are the catalytic subunits of CIII. Ten subunits are encoded by nuclear DNA (*CYCI* (OMIM #615453), *UQCRFS1*, *UQCRQ* (#615159), *UQCRC1*, *UQCRC2* (#615160), *UQCRH*, *UQCRB* (#615158), *UQCRI0*, *UQCRI1*, subunit IX) and cytochrome *b* (*MT-CYB*, OMIM #516020) is encoded by the mitochondrial DNA. There are also a number of associated proteins required for CIII's proper assembly and functioning (Fernández-Vizarra and Zeviani 2015; Gaignard et al. 2013).

Genetic defects that result in CIII-deficiency are among the rarest and most infrequently diagnosed mitochondrial disorders. Until recently only three of the genes causing CIII-deficiency were known (*BCS1L*, *MT-CYB*, and

UQCRB) but recent advances in DNA sequencing technology as well as the continued study of the CIII subunit orthologues in yeast have enabled the discovery of seven additional human genes causing CIII-deficiency (Fernández-Vizarra and Zeviani 2015). The diagnosis of disorders of CIII-deficiency is challenging as they comprise a group of rare conditions that have vastly different phenotypes. The advent of whole-exome sequencing has also facilitated the molecular diagnosis of these rare disorders (Kohda et al. 2016).

In 2013, Gaignard et al. reported two unrelated cases of CIII-deficiency caused by mutations in the gene encoding cytochrome *c₁* (*CYCI*). Cytochrome *c₁* is the heme-containing subunit of CIII and facilitates the transfer of electrons from the Rieske complex to cytochrome *C*. The two reported patients both presented with recurrent episodes of ketoacidosis and insulin-responsive hyperglycemia in the context of intercurrent illness (Gaignard et al. 2013). Both patients also responded well to fluid resuscitation and insulin administration.

Here we describe a case of hyperammonemia, hyperlactatemia, and insulin-responsive hyperglycemia. Exome sequencing revealed homozygous variants in *CYCI*. This is the third report of disease-causing variants in this gene and the first report of a patient presenting in the neonatal period.

Case Report

Our patient is the first child of healthy non-consanguineous individuals of Sri Lankan origin and she presented to our care on the first day of life. Her mother had no previous history of miscarriage. She received prenatal care throughout the pregnancy and there was no history of gestational diabetes. Due to severe intra-uterine growth restriction (IUGR) and oligohydramnios of unknown etiology, the baby was delivered via cesarean section at 34 weeks. Birth weight was 1.4 kg (3rd percentile). The patient required respiratory support at birth for decreased saturation on room air. Initial capillary blood gas demonstrated respiratory acidosis. Throughout the first day of life, the patient continued to be significantly tachypneic with increased anion gap metabolic acidosis, severe lactic acidemia, ketoacidosis, and impressive hyperglycemia (Fig. 1 and Fig. 2a). When she was transferred to our hospital at 17 h of life, she was also found to have an elevated ammonia level of 212 $\mu\text{mol/L}$ (normal 0–55 $\mu\text{mol/L}$).

Acidosis was managed via sodium bicarbonate infusion and hyperammonemia was managed with carnitine, with good response. Continuous intravenous insulin infusion was started at 25 h of life but hyperglycemia remained particularly difficult to manage despite the administration of extremely high doses of intravenous

insulin; up to 0.45 units/kg/h (Fig. 2a, b). Over the next 48 h of life, insulin administration was weaned quickly because of rapid glucose normalization (Fig. 2b). The working diagnosis was transient neonatal diabetic ketoacidosis versus mitochondrial disease. The patient was not dysmorphic and physical exam was unremarkable aside from tachypnea.

Due to her low birth weight, the patient remained in hospital for a total of 6 weeks. At 3 weeks of life, the baby developed *Klebsiella* urinary tract infection and was managed with antibiotics. She remained stable and did not require any further insulin treatment or special diet. Her blood glucose remained between 5 and 7 mmol/L. The patient was subsequently discharged home on levocarnitine, vitamin D, and iron. At 8 months of age, the patient once again presented with a similar episode in the context of viral gastroenteritis. She was admitted to the intensive care unit for correction of severe hyperglycemia (highest glucose 28 mmol/L), hyperammonemia (up to 184 $\mu\text{mol/L}$), and ketoacidosis. She required continuous intravenous insulin infusion (0.1 U/kg/h). The hyperglycemia was quickly corrected and the patient did not require long-term insulin treatment. The patient is now 23 months of age and she suffers from feeding issues such as poor appetite and an aversion to solids. Her diet is supplemented by feeds via nasogastric tube. She also has moderate motor and language delay. She is following her growth curves, though all growth parameters are well below the 1st percentile for age (at 23 months: weight 7.37 kg, height 73.5 cm, and head circumference 45.9 cm).

During the initial metabolic decompensation, the patient underwent an extensive genetic and metabolic work-up, including an array comparative genomic hybridization (aCGH) which was read as normal. The initial metabolic investigation revealed an acylcarnitine profile with increased C2 and C4-OH (related to ketosis) and numerous non-specific elevations in keeping with mitochondrial dysfunction. The plasma amino acids (PAA) profile showed elevated levels of glutamine, alanine, proline, and glycine. The urine organic acids (UOA) profile on day of life 2 was highly suggestive of a mitochondrial disorder. It revealed a massive lactic acid peak as well as elevated acetoacetate, 3-hydroxybutyrate, pyruvate, fumarate, and malic acid. Abdominal ultrasound, skeletal survey, ophthalmologic and hearing exams were all within normal limits. An echocardiogram identified a small secundum atrial-septal defect. Interestingly, brain magnetic resonance imaging showed focal enlargement of the pituitary gland with interval absence of the normal T1 hyperintensity of the neurohypophysis. MR spectroscopy revealed no abnormal peaks. MRI scan was unchanged 1 year later.

Given the patient's unusual presentation, whole-exome sequencing (WES) was deemed the most viable diagnostic

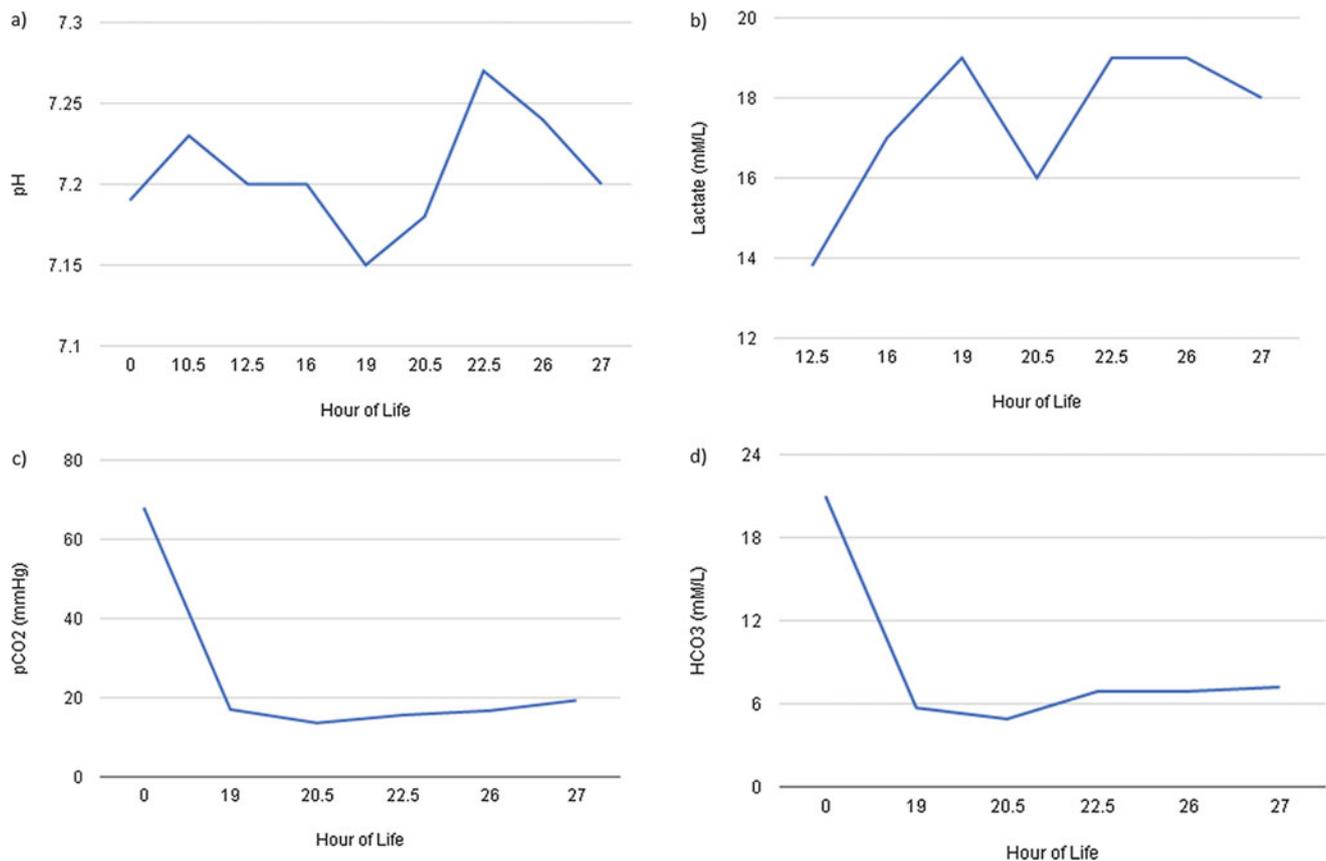


Fig. 1 Patient's (a) pH, (b) lactate, (c) pCO₂, and (d) HCO₃ levels during first 27 h of life

method. The family provided informed consent for participation in the TIDEX gene discovery project (UBC IRB approval H12-00067). WES was performed for the patient and her unaffected parents using the Agilent SureSelect kit and subsequent sequencing was performed on the Illumina HiSeq 2000 (Perkin-Elmer, USA). The sequencing reads (~28X coverage) were aligned to the human reference genome (version hg19) using the Bowtie2 aligner (Langmead and Salzberg 2012) and variants were called using SAMtools (Li et al. 2009). Allele frequencies were assessed in dbSNP142, the Exome Aggregation Consortium (ExAC) database, and an in-house database of more than 350 WES and whole-genome sequencing (WGS) results. Only rare variants ($\leq 1\%$ in dbSNP) were selected for downstream analysis. SNPEff2 (Cingolani et al. 2012) was used to annotate variants and custom-made perl scripts were used to select for consequential variants (missense/nonsense and splice site variants). These variants were subsequently screened under a series of genetic models: homozygous, hemizygous, compound heterozygous, and de novo.

In total, we identified 36 candidate genes affected by rare variants. Of these 36 candidates, investigation of the functionality of the variants and subsequent review of the literature identified the homozygous missense variant

g.145151518C>T (p.Leu215Phe; NM_001916) on chromosome 8 and affecting the *CYCI* gene (MIM 123980) as the most likely functional candidate. The presence of this variant was confirmed by Sanger re-sequencing (Fig. 3). This is a rare, previously reported pathogenic variant (Gaignard et al. 2013) observed with a frequency of $8.251e-06$ in ExAC and not observed in dbSNP (version 142), NHLBI ESP, or our in-house genome database. The variant was predicted to be deleterious by all tested prediction software: a CADD score of 27.1 (Kircher et al. 2014); a SIFT prediction score of 0.001 (cutoff = 0.05) (Kumar et al. 2009); a PROVEAN prediction score of -4 (cutoff = -2.5) (Choi et al. 2012); and a Polyphen2 "probably damaging" score of 1.000 (Adzhubei et al. 2013). This variant has also been classified as pathogenic according to the recently published ACMG Standards and Guidelines (Richards et al. 2015). Furthermore, previously reported experimental results suggest that this variant primarily affects the structural integrity of Cytochrome *c*₁. Variations in this gene have only been previously reported in two other patients with similar presentation. One of these patients is also of Sri Lankan origin and she is homozygous for the same variant found in our patient (Gaignard et al. 2013).

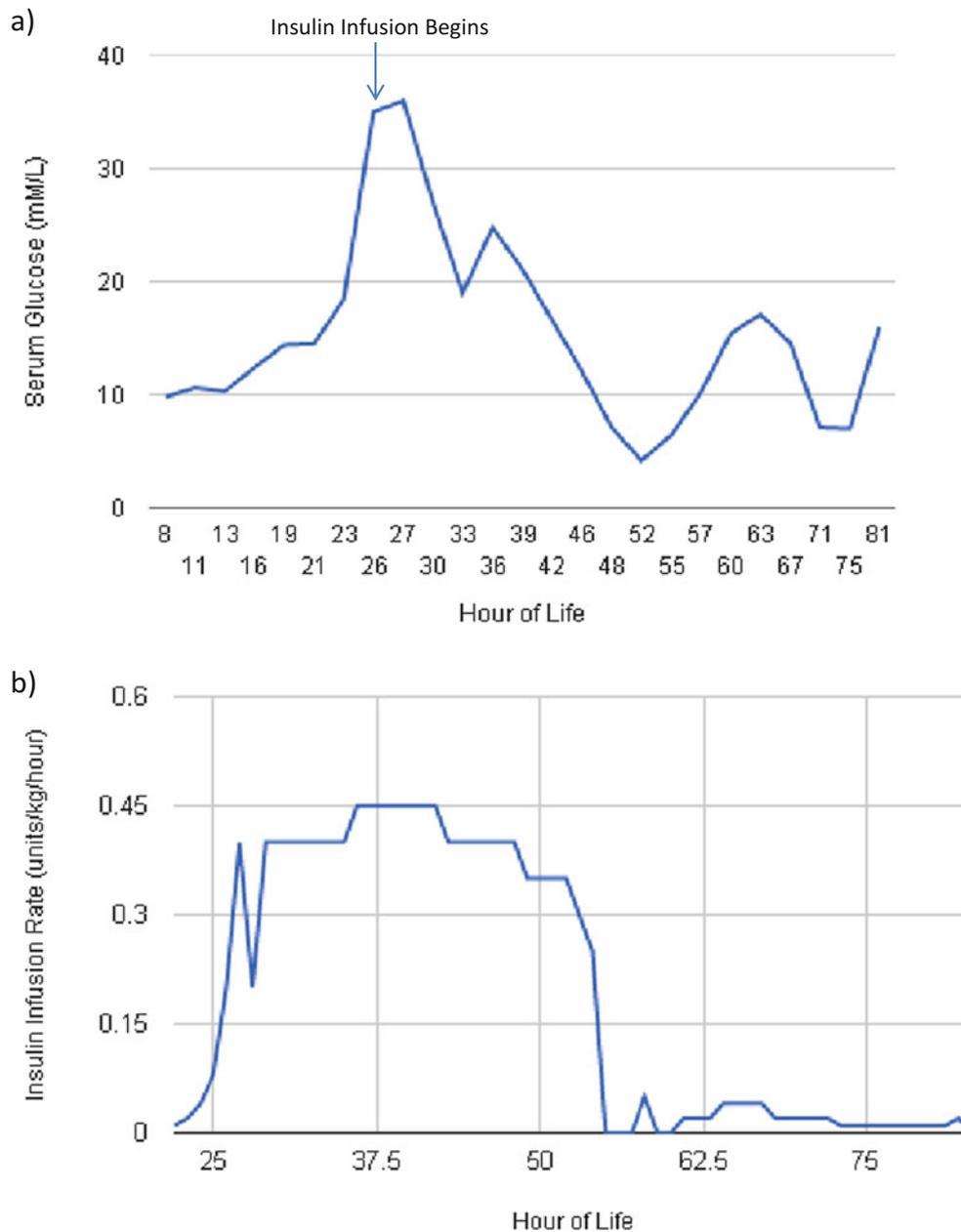


Fig. 2 Patient's (a) blood glucose levels versus hour of life and (b) corresponding insulin infusion rate versus hour of life. Insulin infusion began at approximately 25 h of life

Discussion

Our patient confirms episodic hyperlactatemia, hyperammonemia, and transient insulin-responsive hyperglycemia as the phenotype of CIII deficiency caused by variants in *CYCI*, which encodes the cytochrome c_1 subunit of mitochondrial complex III. However our patient is the first to present in the neonatal period. Our study is limited by the lack of available muscle for respiratory chain enzyme analyses, but it has previously been shown that the c.643C>T variant found in our patient results in reduced

levels of cytochrome c_1 and CIII assembly subunits in the skeletal muscle and fibroblasts of affected patients. These effects are also reproduced when the variant is inserted at the orthologous position in the yeast *CYCI* ortholog. The variant is thought to affect the tertiary structure of the subunit, resulting in increased susceptibility to proteolysis and/or decreased ability to assemble with the other CIII subunits (Gaignard et al. 2013).

Our patient was found to have the same variant as a previously reported Sri Lankan patient, indicating that this variant may be a founder mutation in the Sri Lankan

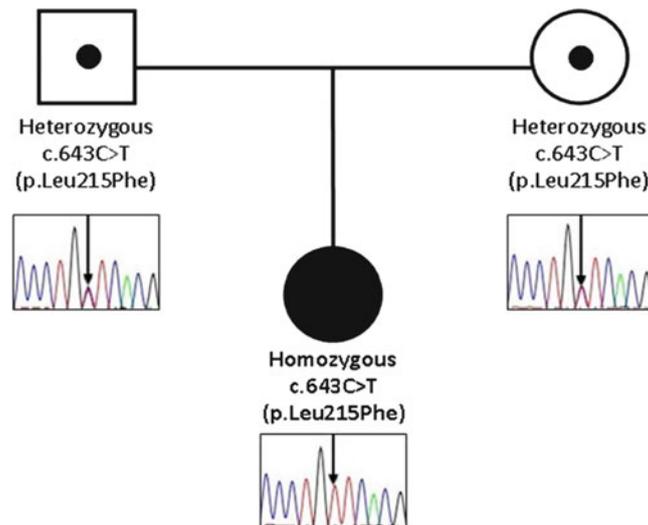


Fig. 3 Family pedigree and Sanger re-sequencing results for parent–patient trio

population. In contrast to the neonatal presentation of our patient, she presented later in childhood, at the age of 2.5 years, with dehydration, vomiting, liver failure, and neurological impairment eventually leading to coma. She was found to have lactic acidosis and hyperammonemia and, similarly to our patient, she improved dramatically with rehydration and insulin administration and did not require long-term insulin treatment. She also suffered numerous subsequent episodes of decompensation, precipitated by minor illness. Feeding issues were not described which contrasts with our patient (Gaignard et al. 2013). At the last reported follow-up at 18 years of age, she was found to have normal psychomotor development.

The other reported patient presented at the age of 5 months with a similar episode of metabolic ketoacidosis after a history of vomiting and febrile illness (Gaignard et al. 2013). He was homozygous for a c.288G>T (p.Trp96Cys) missense variant that was also shown experimentally to primarily affect the structural integrity of cytochrome *c*₁ (Gaignard et al. 2013). Like our patient, he has also suffered numerous episodes of lactic acidosis related to illness and has required insulin therapy during these episodes. This patient had an otherwise negative review of systems and normal development.

Our patient's early manifestations of IUGR, hyperglycemia, and insulin dependency resembled that of transient neonatal diabetes and pancreatic hypoplasia/agenesis (Naylor et al. 2011). However, in the case of transient neonatal diabetes, insulin dependency typically lasts for a few months postnatally and insulin-dependency is lifelong in pancreatic agenesis. Generally, the insulin dose required for these disorders is in the low-dose range: from 0.4 to 0.8 units/kg/day. Our patient required very high insulin

infusion rates: up to 0.45 units/kg/h and unlike patients with transient neonatal diabetes and pancreatic agenesis, she was weaned off insulin quickly – within 48 hours. As previously mentioned, our patient had a normal physical exam at the time of presentation and pancreatic agenesis is usually seen in association with other abnormalities, such as hypothyroidism and immune dysregulation in IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) and biliary or duodenal atresia in Mitchell–Riley syndrome (Mitchell et al. 2004; Concepcion et al. 2014; Duclaux-Loras et al. 2015; Wildin et al. 2002).

As evidenced by our case, the age at presentation and phenotypes of complex III deficiencies can vary significantly, even among patients with the same genotype. This case also reiterates the clinical utility of WES, particularly in disorders with significant clinical and genetic heterogeneity. For the clinician, mutations in *CY1* causing CIII-deficiency may be added to the differential diagnosis of neonatal transient hyperglycemia, lactic acidosis, and insulin dependency. Aggressive rehydration and insulin administration are the keys to recovery.

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Take Home Message

By reading this third case report of a patient with mitochondrial complex III deficiency caused by mutations in the cytochrome C1 gene and the unique way in which

she presented, readers will learn the importance of keeping mitochondrial complex III deficiency in their differential diagnosis given the diverse presentation of patients.

Author Contributions

Nataschia Anastasio, MD, MSc: Planning and drafting of article

MajaTarailo-Graovac, PhD: Analysis and interpretation of data and article revision and contribution

Reem Al-Khalifah, MD: Article contribution and revision

Laurent Legault, MD: Article revision and contribution

Britt Drogemoller, PhD: Analysis and interpretation of data and article revision and contribution

Colin J.D. Ross, MSc, PhD: Analysis and interpretation of data

Wyeth W Wasserman, PhD: Analysis and interpretation of data

Clara van Karnebeek, MD, PhD: Analysis and interpretation of data and article revision and contribution

Guarantor: Daniela Buhás, MD: Analysis and interpretation of data, planning and drafting of article

Compliance with Ethics Guidelines

Conflict of Interest

Nataschia Anastasio, MajaTarailo-Graovac, Reem Al-Khalifah, Laurent Legault, Britt Drogemoller, Colin J.D. Ross, Wyeth W Wasserman, Clara van Karnebeek, and Daniela Buhás declare no conflict of interest.

Patient Consent

Parents provided informed consent for publication of this case report.

Ethical Standards

The authors declare that the experiments comply with the current laws of Canada, the country in which they were performed.

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