Clinical Reasoning: A 2-Day-Old Boy With Sudden Cardiac Arrest and Encephalopathy

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Section 1

A 36-week, 5-day-old boy was born through uncomplicated vaginal delivery to a healthy, 36-year-old G7P4 mother. His resuscitation included tactile warmth, drying, and stimulation, and his Apgar scores were 8 and 9 at 1 and 5 minutes, respectively. He was small for gestational age with a birth weight of 2100 g (0.16 percentile). His course was unremarkable with normal behavior and breastfeeding for approximately 40 hours of life.

On day 2 of life, he became acutely bradycardic to the 60s, apneic, and then pulseless. Resuscitation was performed for 60 minutes with chest compressions, defibrillator shocks, and administration of epinephrine. Notable laboratory test results included a potassium level of 6.8 mmol/L, a WBC count of 19.7×10^9/L, an INR of 2.6, a lactate of 5.2 mmol/L, and a glucose level of 9 mg/dL. After successful resuscitation and administration of a dextrose bolus, his glucose level improved to 245 mg/dL but then rapidly decreased again to 75 mg/dL. He was started on a continuous dextrose infusion, and his subsequent glucose levels normalized.

The patient was intubated and transported to our institution's intensive care nursery for further evaluation. Neurologic examination on admission, while receiving 0.01 mg/kg/h morphine, was notable for somnolence, absent behavioral response to light, diffuse hypotonia, absent spontaneous movements, slight reactive movements with stimulation, hyperreflexia in his upper extremities, and weak suck and Moro reflexes.

Questions for Consideration:

1. What is the differential diagnosis for sudden decompensation in a previously healthy neonate?
2. What diagnostic evaluations should be included to distinguish these possibilities?
3. What empiric therapies would you initiate for this patient?

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Section 2

Our differential diagnosis included infectious, cardiac, neurologic, and metabolic causes for sudden neonatal decompensation.

From an infectious standpoint, he remained afebrile, his peak WBC count was $21.5 \times 10^9/L$, and his blood cultures were negative. A lumbar puncture was deferred given a low suspicion for meningitis.

Cardiac workup included ECGs demonstrating normal sinus rhythm. An echocardiogram showed normal cardiac anatomy with a possible ventricular septal defect and an elevated right ventricular pressure. Troponin was elevated at 0.13 μg/L, and B-type natriuretic peptide was elevated at 1,840 pg/mL. Together, these data supported a cardiomyopathy but whether cardiomyopathy was the primary cause of, or a secondary result of, the cardiac arrest was unclear.

Neurologically, his EEG showed nonspecific findings of mild excessive discontinuity and moderate voltage attenuation (figure 1, A and B), which normalized over 24 hours (eFigure 1A and B available from Dryad: 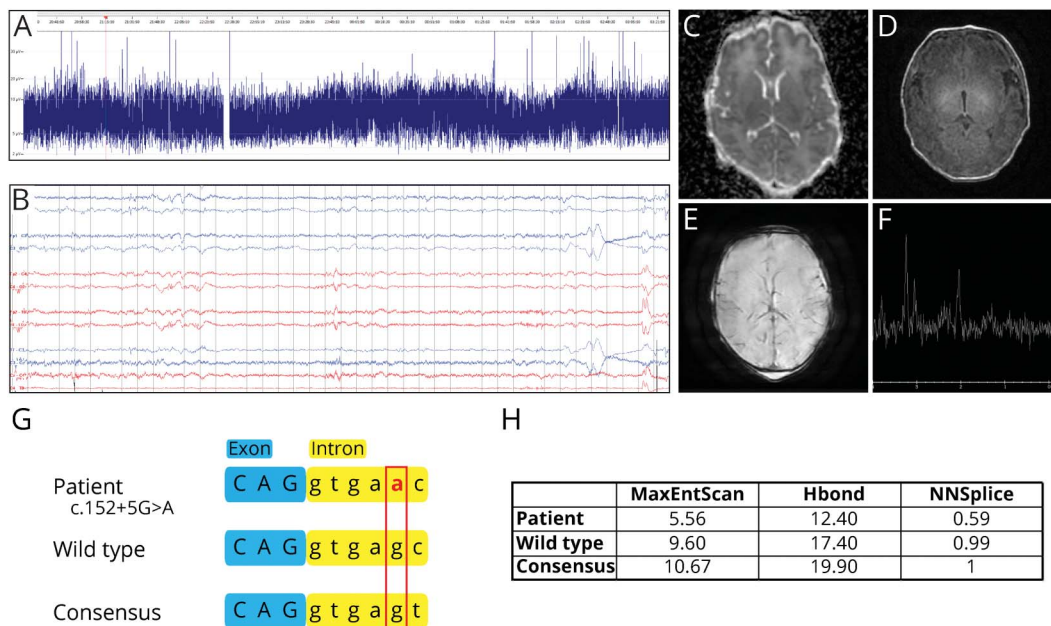doi.org/10.7272/Q6JQ0Z8F); there were no epileptiform discharges or seizures. At day 5, his encephalopathy improved and he had only mild hypotonia. A brain MRI with spectroscopy performed on day 6 was unremarkable (figure 1, C–F) and without evidence of hypoxic-ischemic injury.

The patient's small size for gestational age, severe hypoglycemia, cardiomyopathy, and sudden decompensation in an otherwise healthy infant led us to consider metabolic disorders as a unifying diagnosis; however, initial metabolic studies were unrevealing. His liver function tests and ammonia were normal, and his elevated BUN of 31 mg/dL and lactate of 5.2 mmol/L were more likely a consequence of his acute cardiac arrest. Thus, more targeted metabolic testing was warranted.

Questions for Consideration:

1. Is there a unifying diagnosis or category of diagnoses you would consider?
2. Are there additional metabolic studies you would send at this time?
3. Given the results of his neurologic workup, what can you infer about the severity of his anoxic brain injury?

Figure 1 The Patient's EEG, Radiologic, and Genetic Data

	MaxEntScan	Hbond	NNSplice
Patient	5.56	12.40	0.59
Wild type	9.60	17.40	0.99
Consensus	10.67	19.90	1

(A) Amplitude-integrated EEG demonstrated a discontinuous background with attenuated voltages but without seizures. (B) Full bipolar montage EEG demonstrating discontinuity with interburst intervals of about 8 seconds, but no epileptiform discharges or seizures. EEG was performed while the patient was receiving 0.01 mg/kg/h morphine. (C–F) Axial sections from the patient's brain MRI demonstrating a normal ADC map (C) without evidence of acute injury, a normal T1-weighted sequence (D) without evidence of injury or structural malformations, and a normal susceptibility-weighted sequence (E) without evidence of intracranial hemorrhage. (F) The patient's MR spectroscopy sampled from the basal ganglia demonstrates no abnormal peaks to suggest acute injury or metabolic derangement. (G) Schematic diagram demonstrating the sequence variation of our patient in comparison with wild-type and consensus splice site sequences. The 152+5G>A variant is contained within the 5′ splice site consensus sequence encoding a G → A transversion at nucleotide 5 from the intron start site (bold red font). The wild-type sequence is the most frequent allele found in healthy populations. The consensus sequence is a strong variant of the canonical sequence MAG|GURAGU, where M is adenine or cytosine and R is a purine. (H) Summary data of in silico models used to predict the strength of the patient's splice site: MaxEntScan uses the principle of maximum entropy to score the strength of a splice site. HBond calculates the number of hydrogen bonds formed between the splice site and the U1 snRNA. NNSplice is a neural network trained on established consensus splice sites. In all cases, a higher score indicates a stronger potential splice site, and in all cases, the patient's genetic variant scored substantially lower than the wild-type or consensus sequences. These models have been previously validated as robust predictors of cryptic splicing[12] and are freely available to the public.

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Section 3

The patient rapidly improved as evidenced by his 24-hour follow-up examination, EEG, and brain MRI. Taken together, this data suggest that he did not suffer significant cerebral hypoperfusion and imply that he was likely not pulseless for the full 60-minute code.

To expand his metabolic evaluation, further screening studies were ordered. His plasma amino acid analysis was unremarkable, but his urine organic acid analysis identified elevated lactate and medium- and long-chain fatty acids. His plasma acylcarnitine profile and the California state newborn screen revealed an elevation of multiple long-chain acylcarnitine species, including dramatic elevations of C16 and C18:1 acylcarnitines. This pattern is characteristic of 2 long-chain fatty acid oxidation disorders—carnitine palmitoyltransferase 2 (CPT2) deficiency and carnitine-acylcarnitine translocase (CACT) deficiency, which cannot be distinguished biochemically. For genetic confirmation, an InVitae Fatty Acid Oxidation Defects Panel was sent, which identified 2 genetic variants: (1) heterozygosity of c.625G>A (p.Gly209Ser) in *ACADS*, which is a benign and common variant,[1,2] and (2) homozygosity of c.152+5G>A (intronic) in *CPT2*, which is a variant of uncertain significance and was presumed to be pathogenic (figure 1G).

Given the strong clinical, biochemical, and genetic evidence of CPT2 deficiency, the infant was initiated on a long-chain fat-restricted diet, supplemented with levocarnitine and medium-chain triglyceride (MCT) oil, and started on triheptanoin, a newly FDA-approved medium-chain triglyceride.[3,4]

Discussion

CPT2 deficiency is an autosomal recessive disorder of fatty acid metabolism characterized by recurrent attacks of myalgias and myoglobinuria.[5] The disease manifests as 3 distinct phenotypes: (1) a mild myopathic form that starts in childhood or adulthood, (2) a severe multisystem infantile form, and (3) a lethal neonatal form.[5] In the mild myopathic form, patients may present in the second or third decade of life with exercise-induced weakness, myalgias, and rhabdomyolysis, the latter of which can cause acute renal failure.[5] Conversely, the lethal neonatal form is exceedingly rare, and affected individuals present with nonketotic hypoglycemia, hypotonia, cardiomyopathy, cardiac arrhythmias, and seizures.[6] Patients often die within the first months of life because of cardiomyopathy or hypoglycemia-associated cardiac arrhythmias or seizures.[6,7] Our patient suffered a life-threatening cardiac arrest, which was attributed to a cardiac arrhythmia secondary to severe hypoglycemia and hyperkalemia.

Biochemically, CPT2 deficiency leads to tissue energy failure through aberrant shuttling of acylcarnitines to the beta-oxidation pathway. The carnitine shuttle is a cellular pathway that delivers long-chain fatty acids from the cytosol to the mitochondria. Long-chain fatty acids are transported as acylcarnitines through the sequential actions of acyl-CoA synthetase, carnitine palmitoyltransferase I (CPT1), and carnitine-acylcarnitine translocase (CACT). Once in the mitochondria, these long-chain fatty acids are reconverted into their original acyl-CoAs by carnitine palmitoyltransferase II (CPT2) to supply the precursors for beta-oxidation. Disruption of this pathway leads to failed production of acetyl-CoA for ketone generation as well as $FADH_2$ and $NADH^+$ that are required for oxidative phosphorylation.[8]

Diagnostically, CPT2 deficiency is inferred through newborn screen blood spots or plasma acylcarnitine profiles using mass spectrometry. This test shows the characteristic elevation of long-chain acylcarnitines, especially C16:0 and C18:1, and a concomitant decrease in C2.[5] Our patient had a 55.4-fold and 22.1-fold increase in C16:0 and C18:1, respectively, and a 3.5-fold decrease in C2 (eTable available from Dryad: doi.org/10.7272/Q6JQ0Z8F). CPT2 deficiency results in an identical acylcarnitine pattern to CACT deficiency, and therefore, genetic analysis is required to differentiate these 2 diseases.

The InVitae Fatty Acid Oxidation Defects Panel found that our patient harbors a novel and previously unreported homozygous variant within intron 1 of *CPT2* (figure 1G). To predict the effects of our patient's variant on gene splicing, we used 3 in silico modeling algorithms that assess the strength of the modified splice site.[9-11] All 3 models predicted weak splice site activity relative to the wild-type sequence (figure 1H). The functional consequences of such variants are disruption of the native U1 snRNP binding to the consensus splice site, which results in the unmasking of alternative splice sites in other regions of the transcript. This process, referred to as *cryptic splicing*, leads to the aberrant production of nonphysiologic mRNA splice variants.[12,13] Taken together, this supports a pathologic role for this patient's homozygous 152+5G>A variant in *CPT2*, likely through the production of a cryptic splice site. Functional validation at the transcript and protein level is needed to validate this hypothesis.

Notably, only 1 prior report has identified an association between consensus splice sequence variants and neonatal CPT2 deficiency.[14] In this cohort, all affected individuals were compound heterozygotes for an indel mutation and a 3′ splice site mutation. Thus, we report a novel case of CPT2 deficiency due to homozygosity of a splice site variant alone.

Unfortunately, treatment options for CPT2 deficiency remain limited. Therapies focus on avoidance of metabolic decompensation by avoiding prolonged fasting, restricting long-chain fatty acid intake, supplementing medium-chain fatty acids and carnitine, and encouraging frequent carbohydrate-rich meals. In 2020, the FDA approved the use of triheptanoin, a 7-carbon medium-chain triglyceride, for the treatment of long-chain fatty acid oxidation disorders. Triheptanoin is metabolized independently of the cellular pathway for long-chain fatty acid oxidation, and clinical trials demonstrate that treated patients are less likely to develop hypoglycemia, cardiomyopathy,

rhabdomyolysis, and hepatomegaly.[2,3] However, this drug has not been tested in the neonatal form of CPT2 deficiency.

Understanding the molecular basis of CPT2 deficiency is crucial to inspire future therapeutic discoveries. It is intriguing to speculate that splice site–mediated CPT2 deficiency could be treated with splice-modulating antisense oligonucleotides as has been done in spinal muscular atrophy and Duchenne muscular dystrophy.[15] Given the ever-increasing use of newborn screening and genetic profiling, it may indeed be possible to engineer patient-specific targeted therapies for early intervention of these devastating metabolic disorders.

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Disclosure

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Name	Location	Role	Contribution
Brandon Holmes, MD, PhD	University of California San Francisco, CA	Author	Designed and conceptualized the study, participated in patient care, analyzed data, and drafted and revised the manuscript
Jeffrey Russ, MD, PhD	University of California San Francisco, CA	Author	Designed and conceptualized the study, participated in patient care, analyzed data, and drafted and revised the manuscript

Appendix (continued)

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