Cold Spring Harbor Molecular Case Studies - Research Report

Full Title – **Ethylmalonic encephalopathy masquerading as meningococemia**

Short Title – **Ethylmalonic encephalopathy as a masquerade**

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ABSTRACT (250 Words)

Ethylmalonic encephalopathy (MIM #602473) is a rare autosomal recessive metabolic condition caused by biallelic variants in *ETHE1* (MIM #608451), characterized by global developmental delay, infantile hypotonia, seizures and microvascular damage. The microvascular changes result in a pattern of relapsing spontaneous diffuse petechiae and purpura, positional acrocyanosis and pedal edema, hemorrhagic suffusions of mucous membranes and chronic diarrhea. Here we describe an instructive case in which ethylmalonic encephalopathy masqueraded as meningococcal septicemia and shock. Ultra-rapid whole genome testing (time to result 60 hours) and prompt biochemical analysis facilitated accurate diagnosis and counselling with rapid implementation of precision treatment for the metabolic crisis related to this condition. This case provides a timely reminder to consider rare genetic diagnoses when atypical features of more common conditions are present, with an early referral to ensure prompt biochemical and genomic diagnosis.

INTRODUCTION

Undifferentiated presentations in acute pediatrics form the backbone of clinical practice with evidenced-based care pathways used to improve outcomes and support timely patient care. Meningococemia and its timely management are sentinel cases for a healthcare system and the quality of care provided. This severe infection typically presents with septic shock and a characteristic purpuric rash that is often considered pathognomonic for the condition. Rapid administration of antimicrobial and supportive therapies is best practice; however, cases with atypical features or that fail to respond to standard treatment require consideration of alternate diagnoses.

Although individually uncommon, rare genetic diseases collectively account for 15.9% of pediatric hospital admissions(Gjorgioski et al. 2020), and early recognition may allow the introduction of targeted therapies. The introduction of ultra-rapid genomic sequencing in the diagnosis of seriously ill neonates and children is revolutionizing the early detection of rare genetic and metabolic diseases, resulting in some cases in the early institution of life-saving interventions.(Lunke et al. 2020; Dimmock et al. 2021; Owen et al. 2021; Stark and Ellard 2021) We present a case of a rare metabolic condition, ethylmalonic encephalopathy (MIM #602473), masquerading as meningococemia with atypical features. This case provides a timely reminder to consider rare genetic diagnoses when atypical features of more common conditions are present, with an early referral for a prompt genomic and biochemical diagnosis being key to management.

RESULTS

Clinical Presentation and Family History

The patient, IV.I, is a 9-month-old female who presented with an acute deterioration, involving depressed conscious state, hypotonia, reduced peripheral perfusion with only central pulses palpable, and a rapidly evolving widespread purpuric rash (**Figure 1**). She was initially assessed at home by paramedics who considered the rash to be pathognomonic for meningococcal septicemia. The patient's spontaneous respiratory effort was supported with bag-valve-mask and oxygen, intra-osseous access was placed, ceftriaxone and hydrocortisone administered, and a fluid bolus commenced during time-critical transport to the pediatric emergency department. Upon arrival to the pediatric emergency department, the patient was hypothermic (33.7°C), minimally responsive, poorly perfused, with a marked metabolic acidosis (lactate 6.6 mmol/L, normal range 0.5-2.0). Peripheral inotropes were commenced, and the patient intubated and transferred to Pediatric Intensive Care for stabilization. There were no prolonged periods of hypoxia during hospital admission. Investigations on admission are shown in **Table 1**.

Table 1. Presenting Features: Initial Investigation Results

	Normal range	Day 0	Day 1
Blood gas			
pH	7.32-7.43 ^V 7.35-7.45 ^A	7.31 ^V	7.33 ^A
pCO ₂	38-61 ^V mmHg 32-45 ^A mmHg	23 ^V	44 ^A
HCO ₃	22-32 ^{V,A} mmol/L	12 ^V	23 ^A
Base excess	-3-+3 ^{V,A} mmol/L	-15 ^V	-3 ^A
Lactate	0.5-2.0 ^{V,A} mmol/L	6.6 ^V	1.4 ^A
Glucose	3-7.7 ^{V,A} mmol/L	21.6 ^{V*}	5.3 ^A
Full blood picture			
Hb	100-140 g/L	126	86
WCC	5-18 x10 ⁹ /L	22.5 [*]	11.6

Neutrophils	1.5-9 x10 ⁹ /L	9.72 *	8.10
Platelets	150-450 x10 ⁹ /L	618 *	441
Markers of muscle breakdown			
CK	30-150 U/L	68	-
Troponin I (HS)	0-40g/L	137 n	-
Coagulation profile			
INR	0.8-1.2	1.2	1.2
APT	22-32 secs	26 secs	25
Fibrinogen	1.5-4.0 g/L	1.5	2.9
Inflammatory markers			
CRP	0-5 mg/L	0.5	13
Procalcitonin	0.00-0.07 mcg/L	0.32	-
Ferritin	20-310 mcg/L	29	29
CSF			
RCC	x10 ⁶ /L	1240	760
Polymorphonuclear cells	x10 ⁶ /L	4	2
Protein	0.15-0.45 g/L	0.32	0.33
Glucose	2.5-5.0 mmol/L	8	3.9
Lactate	1.2-2.8 mmol/L	5.5	2.6
Molecular microbiology			
Meningococcal PCR	Not detected	Negative	Negative
Serum			
Meningococcal PCR CSF	Not detected	Negative	Negative
Enterovirus PCR CSF	Not detected	Negative	Negative
SARS-CoV-2 PCR	Not detected	Negative	Negative

Hb hemoglobin; WCC white cell count; CRP C-reactive protein; RCC red cell count; CSF cerebrospinal fluid; PCR polymerase chain reaction; V Venous; A arterial.

*Post hydrocortisone

Supportive management in the pediatric intensive care unit (PICU) over the next 48 hours included inotropic support, fluid resuscitation and ventilation together with empiric antimicrobial agents. In contrast to what was expected, markers of inflammation (CRP and procalcitonin) remained low, neutrophil and platelet count high with no evidence of disseminated intravascular coagulopathy, and needle aspiration of the purpuric rash was negative for bacteria on gram stain. Following a normal head CT, a lumbar puncture was performed 20 hours after admission and repeated on Day 2, both with CSF samples demonstrating low cell counts (**Table 1**). Supportive care resulted in improvement in the metabolic acidosis and correction of lactic acidosis. An echocardiogram demonstrated a structurally normal heart with dilated left ventricle with mildly depressed systolic function, as commonly seen in critically unwell patients in the setting of an acidosis. Echocardiographic findings normalized within four days. Inotropes were ceased and the patient was extubated 48 hours post presentation. No organism was isolated on blood, urine, or CSF cultures. Autoimmune markers including ANA, ANCA, dsDNA, RF antibodies were normal.

Magnetic resonance imaging (MRI) brain performed on Day 2 demonstrated an unusual pattern of acute on chronic changes (**Figure 2**). Reduced size of the left cerebral hemisphere, bilateral T2 hyperintense and T1 hypointense periventricular lesions without restricted diffusion were consistent with chronic, potentially *in utero*, diffuse ischemic damage to the left cerebral hemisphere white matter and more focal chronic infarcts in the periventricular white matter, respectively, similar to the appearances of typical pre-term ischemic white matter injury. In addition, however, we identified bilateral punctate T2 hyperintense lesions demonstrating true restricted diffusion involving the basal ganglia and thalami, with some lesions demonstrating susceptibility-related signal loss consistent with hemorrhagic infarcts. This biphasic appearance of acute and established ischemic pathology and hemorrhagic infarcts was not consistent with meningococemia but rather a non-infective, potentially genetic, vasculitis, with provisional diagnoses of *ADA2* or *STING1*-related vasculitis proposed based on the MRI abnormalities, especially given the cutaneous rash and systemic clinical presentation. With increasing suspicion of a non-infective cause to explain the patient's condition, referrals to neurology, clinical genetics, and metabolic medicine were made on Day 4/5 of admission. Electroencephalogram (EEG) on Day 6 demonstrated encephalopathy without overt seizure activity.

Further information was sought at this time about the family history. The patient's parents were consanguineous (first cousins) of Afghani ethnicity (see pedigree, **Figure 3**). The mother had two previous pregnancies. Her first pregnancy ended in a termination of pregnancy at 26 weeks gestation for an antenatal diagnosis of arthrogryposis. Genetic testing at this time identified a previously reported (Sandaradura et al. 2018) homozygous *TNNT3* (MIM #600692) variant, NM_006757.3(TNNT3):c.681+1G>A which explained the fetus' clinical presentation with a diagnosis of distal arthrogryposis (MIM #618435). Subsequent testing of the parents confirmed that both were unaffected carriers for this variant. Her second pregnancy ended in the live birth of a male infant, who is now 3 years and remains well, with reported normal neurodevelopment. The mother's third pregnancy ended in the live birth of the current patient. She had genetic testing for the *TNNT3* variant antenatally revealing unaffected status. Her neurodevelopment was delayed in comparison to her brother, with hypotonia, and delayed gross and fine motor on review of family videos. In the previous months there had been instances of profound positional acrocyanosis (**Figure 4**). On the day of presentation, her parents noticed sustained clonus (**supplemental video 1**). There was no other family history of abnormal neurodevelopment or other neurological conditions.

Given the history of hypotonia, symmetry of MRI findings, encephalopathy, purpuric and petechial rash, and recurrence of lactatemia in the absence of acidosis or perfusion concerns on Day 3, a genetic or metabolic cause for the patient's presentation was considered most likely, and she was approved for inclusion in a rapid genomic diagnosis program, the Australian Genomics Acute Care study (Lunke et al. 2020), on Day 4 of admission. Following genetic counseling, the parents provided written consent for trio whole genome sequencing (WGS) and samples collected on Day 5 of admission. Urine metabolic investigations were requested and performed in tandem with ultra-rapid WGS sequencing.

Genomic Analyses

Whole genome sequencing results

Results of ultra-rapid WGS were reported on Day 6, 60 hours after receipt of samples, and identified a homozygous frameshift variant in exon 2 of *ETHE1* (MIM #608451), c.131_132del; p.(Glu44Valfs*62) (**Table 2**). This variant was classified according to modified ACMG/AMP criteria (Richards et al. 2015). The variant c.131_132del results in a frameshift and is predicted to cause nonsense mediated decay (NMD) and loss of protein with a premature termination codon (PVS1). Loss of function is an established mechanism of disease with multiple other splice site, frameshift, nonsense and missense variants classified as likely pathogenic and pathogenic in ClinVar. This variant has been previously reported in homozygous and compound heterozygous state in individuals affected with ethylmalonic encephalopathy (Tiranti et al. 2009; Dionisi-Vici et al. 2016; Boyer et al. 2018) (PM3_strong), with cosegregation demonstrated in one family.(Tiranti et al. 2009) The variant is present in a large population database at an allele frequency of <0.01 for a recessive condition (3 heterozygotes, 0 homozygotes, highest population specific allele frequency 0.000054, East Asian 1/18380) (gnomAD v2.1.1 <https://gnomad.broadinstitute.org/> (accessed 2-Oct-2021) (PM2_supporting). The variant was confirmed by trio analysis to be maternally and paternally inherited (biallelic). Based on modified ACMG/AMP criteria (Richards et al. 2015), this variant was classified as pathogenic. This result is consistent with a diagnosis of ethylmalonic encephalopathy (MIM #602473) and is concordant with the results of the urine metabolic screen and organic acids analysis (**Table 3, see below**).

Table 2. WGS Results – Variant Table

<u>Gene</u>	<u>Chromosome</u>	<u>HGVS DNA</u>	<u>HGVS Protein</u>	<u>Variant</u> <u>Type</u>	<u>Predicted</u> <u>Effect</u>	<u>gnomA</u> <u>D</u>	<u>Zygosity</u>	<u>Inheritance</u>	<u>dbSNP ID</u>	<u>ClinVar</u> <u>ID</u>	<u>Classification</u>
<i>ETHE1</i>	Chr19	NM_014297.5: c.131_132delAG	p.(Glu44Valfs*62)	Frame -shift	Premature truncation codon	3/25104 0	Homo- zygous	Biallelic	rs7618277 30	504493	Class 5; Pathogenic

Phenotypic Analyses

Urine Metabolic Screen

The urine metabolic screen and organic acids analysis (**Table 3**) demonstrated a pattern consistent with either multiple acyl-CoA dehydrogenase deficiency (MADD) or ethylmalonic encephalopathy (C4-C6 acylcarnitines/acylglycines, ethylmalonic acid, isovaleryl glycine, isobutyryl glycine, 2 methylbutyryl glycine, and methylsuccinic). Urine thiosulfate levels were subsequently analyzed and found to be elevated. Urine thiosulfate is a marker of abnormal hydrogen sulfide metabolism, which is more specific for ethylmalonic encephalopathy and not expected to be increased in MADD.

Table 3. Biochemical Analyses

Urine Metabolic Screen	
	Result
C4 carnitine	++
C4 glycine	+++
C6 glycine	++
C5 dicarboxylic	++
C5 carnitine	+
C5 dicarboxyl carnitine	+
C5 glycine	+
Urine Organic Acid and Acylglycine Screen	
Adipic acid	+
Ethylmalonic acid	>+++
Hexanoyl glycine	>+++
Isobutyryl glycine	>+++
Isovaleryl glycine	++
2-Methylbutyryl glycine	+
Methyl succinic acid	++
Urine Metabolites	
Thiosulfate	High

Newborn Screening Results (retrospective)

Following receipt of the results from the urine metabolic screen and WGS, the patient's newborn screening bloodspot screening analysis was retrospectively reviewed (**Figure 5**). This showed increased C4 and C5 carnitine, relative to controls, consistent with ethylmalonic encephalopathy. However, the values were below the decision limits for a sample recollection and the sample was therefore not flagged for further action at the time.

Patient Follow-Up and Clinical Outcomes

The initial results of the urine metabolic studies showed increases in several metabolites (ethylmalonic, hexanoyl glycine, glutaryl carnitine) that initially suggested the possibility of multiple acyl CoA dehydrogenase deficiency. The patient was started on riboflavin, co-enzyme Q10 and carnitine enterally on Day 5, with intravenous N-acetyl cysteine (NAC), metronidazole and a low protein intake added to reduce the enteric hydrogen sulfide (H₂S) production and levels on Day 6. Increased ethylmalonic acid excretion is not specific for ethylmalonic encephalopathy, as it is also increased in short chain acyl-CoA dehydrogenase deficiency (MIM #201470) and several genetic disorders causing multiple acyl-CoA dehydrogenase deficiency. Increased thiosulfate appears to be a reliable means of distinguishing ethylmalonic encephalopathy from these other disorders with increased ethylmalonic excretion (Blau et al. 2014). Targeted analysis of thiosulfate (a metabolite of hydrogen sulfide) in the urine was performed with results received after genomic sequencing.

Despite the targeted medical management, the patient's lactate levels rose from normal levels on Day 3 with a subsequent decline in conscious state requiring re-intubation on Day 4. Between Day 3 and Day 5 a persistent lactemia of between 6 and 11.5 mmol/L (normal 1.2-2.8 mmol/L) reflected ongoing metabolic crisis. On Day 8 of admission, she was noted to have fixed dilated pupils with progressive loss of brainstem function over the following 24 hours. Head CT demonstrated diffuse supratentorial hypoxic ischemic injury with supratentorial edema and transtentorial herniation. A nuclear medicine scan on Day 9 confirmed brain death. A specific trigger for the metabolic decompensation was not identified.

The family will have further follow-up and both grief and genetic counselling. The family has already indicated that they intend on having further children, as such these results will have major utility for informed counselling and decision-making for the family.

DISCUSSION

We present a case of a rare metabolic condition, ethylmalonic encephalopathy, masquerading as meningococcal septicemia, a common and life-threatening pediatric condition. This case provides a timely reminder to consider rare genetic diagnoses when atypical features of more common conditions are present – such as her normal inflammatory markers, negative cultures, ongoing lactic acidemia without metabolic acidosis and fluctuating conscious state. Shared and unique features of meningococcal septicemia and ethylmalonic encephalopathy are summarized in **(Table 4)**. Chronic symmetric MRI brain changes and preceding developmental and neurological concerns, together with a history of consanguinity, were suggestive of an underlying metabolic disorder and increased the probability of a genomic diagnosis. The specific findings on the urine metabolic and organic acid screens, focused the genomic analysis, with ultra-rapid genomic sequencing confirming the diagnosis of ethylmalonic encephalopathy for this family.

Table 4. Comparison of Meningococcal Septicemia and Ethylmalonic Encephalopathy

	Meningococcal Septicemia	Ethylmalonic Encephalopathy
Shock	+	+/-
Petechiae	+	+
Purpura	+	+
Peripheral perfusion	Poor	Poor
Positional acrocyanosis	-	+
Lactate/Metabolic Acidosis	High, normalizes	High, persistent
DIC	+/-	-
Altered conscious state	+	+
Seizures	+/-	+/-
Altered respiratory status	+	+
Diarrhea	+/-	+/-
Inflammatory markers	High	Normal/Low
White cell count	High	Normal
Response to antimicrobials	Rapid	No
Abnormal CSF Cell Count/Biochemistry	Yes	No
Meningococcal PCR or culture positive in blood or CSF	+/-	No
MRI brain changes	Acute	Acute on Chronic/Chronic
Abnormal neurodevelopmental history	Unlikely	Likely
Abnormal neurological signs	Follows rash	Precedes rash
Abnormal urine metabolic screen	Unlikely	Likely

Ethylmalonic encephalopathy (MIM #602473) is a rare autosomal recessive metabolic condition, caused by biallelic variants in *ETHE1* (MIM #608451). It is associated with global developmental delay, generalized infantile hypotonia, chronic diarrhea, seizures, and microvascular damage. The microvascular changes result in a pattern of diffuse and spontaneous relapsing petechiae and purpura, orthostatic acrocyanosis and pedal edema, together with hemorrhagic suffusions of mucosal surfaces. It is characterized by a buildup of hydrogen sulfide and thiosulfate toxic metabolites. Neurologic deterioration often accelerates following illnesses and metabolic crises, with children usually dying in the first decade. Natural history and prognosis are altered by the timing of initiation of therapies such as supportive therapy, NAC, metronidazole, and tailored diets. Early initiation of NAC, metronidazole and modified diet has been shown to alter neurodevelopmental trajectory as well as reduce the frequency of crises.(Viscomi et al. 2010; Boyer et al. 2018) There is preliminary evidence for continuous renal replacement therapy (CRRT) for thiosulfate clearance and to assist in a crisis.(Cardelo Autero et al. 2021) Although it does not reverse prior cerebral damage, liver transplantation stabilizes, or even improves, the disease course in ethylmalonic encephalopathy, reducing the frequency and severity of crises, and representing an opportunity for intervention after early detection before irreversible neurological damage occurs.(Tam et al. 2019; Zhou et al. 2020; Olivieri et al. 2021)

The diagnosis is suggested by clinical findings and substantiated by biochemical testing showing increased serum lactate, C4-C5 acylcarnitine esters, plasma, and urine thiosulfate and urinary ethylmalonic acid. Documented mild cases, late-onset cases and those with only intermittent biochemical evidence are reported.(Di Rocco et al. 2006; Kitzler et al. 2019; Ersoy et al. 2020) Brain MRI often demonstrates symmetric patchy T2-weighted signals in the basal ganglia, periventricular white matter and dentate nuclei, brain stem, and cerebellar white matter.(Di Meo et al. 1993; Tiranti et al. 2004) In some instances, cortical atrophy and diffuse leukoencephalopathy are present.(Di Meo et al. 1993; Tiranti et al. 2004)

Genetic conditions result in prolonged length of stay, increased case complexity as well as increased readmissions.(Gjorgioski et al. 2020) As the proportion of pediatric patients admitted to hospitals with underlying genetic causes increases, and case complexity and total hospitalization costs increase,

ultra-rapid genomic testing will become an increasingly important tool to achieve diagnoses in a time- and cost-efficient manner. Considering rare genetic conditions, especially in patients whose clinical presentation is atypical, will enable earlier test initiation, and avoidance of unnecessary investigations and treatments. Whilst atypical presentations of common conditions are often more likely than rare genetic conditions, the clinical impact of a diagnosis can be significant and early genomic diagnosis may be lifesaving, particularly for metabolic conditions. These often present with crises and critical illness on the background of mild chronic concerns and may occur with minor environmental triggers. Metabolic disorders often have specific emergency management plans and as such diagnosis is critical to timely and targeted therapy.

Despite the extremely rapid biochemical and genomic diagnosis and appropriate treatment, the outcome for this patient and family was still poor, prompting us to consider the utility of screening approaches in the reproductive and newborn settings. Considering the large proportion of consanguineous relationships in some communities it is important to remember that there is a residual risk of ~3% for additional recessive diseases after an initial genetic diagnosis, such as in this family.(AlAbdi et al. 2021) This further raises the question about improved access for families to reproductive carrier screening both before and after the birth of a child with an autosomal recessive disease, especially in the context of consanguinity. *ETHE1* is included in most comprehensive reproductive carrier screening platforms and could have been avoided if it was more widely available to couples, especially those at higher risk. In contrast *TNNT3* is not included on most platforms. A low-risk reproductive carrier screening result does not exclude a rare genetic condition, and as such clinicians should remain vigilant.

Ethylmalonic encephalopathy is potentially identifiable by newborn bloodspot screening (NBS) using C4/C5 carnitine levels. This is illustrated by an unrelated child with ethylmalonic encephalopathy and C4/C5 carnitine levels above NBS decision limits (P2 in Figure 5). P2 presented clinically in the first few days of life, suggesting that later onset patients, such as the one presented here, may be more difficult to detect on NBS due to lower metabolite levels. Ethylmalonic encephalopathy is not currently included on NBS in many countries, influenced by its low prevalence and lack of a definitive treatment. However, evidence for the impact on morbidity including improved neurodevelopment and

reduced metabolic crises suggests that this may soon change.(Boyer et al. 2018) Early administration of NAC, metronidazole and specific diets results in improved clinical outcomes and biochemical markers.(Boyer et al. 2018) In the case of the proband, IV.I, the NBS C4/C5 levels were increased but did not reach the decision limits for recollection. This reinforces the need to remain vigilant for metabolic conditions in older infants and children despite a normal NBS result. Data on the sensitivity of NBS for ethylmalonic encephalopathy are lacking; cases such as ours suggest that at least some cases will be missed on NBS. This is in keeping with other metabolic conditions, in particular those that require stress or a trigger to cause abnormalities in metabolic analytes.(Elmonem and van den Heuvel 2021) Despite the limitations of NBS to detect ethylmalonic encephalopathy, retrospective analysis of NBS results from high-risk cases, such as this one, may provide useful diagnostic or confirmatory evidence. NBS for several inborn errors of metabolism is currently based on tandem mass spectrometry, but research and debate are currently ongoing on the potential transition to genomic sequencing for NBS as an opportunity for population health screening initiatives(Woerner et al. 2021) which may result in earlier diagnosis and treatment of rare inborn errors of metabolism such as ethylmalonic encephalopathy that are not routinely included on current NBS panels.

In summary, this case provides a timely reminder to consider rare genetic diagnoses when atypical features of more common conditions are present, with an early referral for a prompt genomic diagnosis. It also raises questions regarding the timing and utility of genomic sequencing as part of newborn and reproductive carrier screening programs.

METHODS

Genomic Sequencing

Genomic sequencing was performed by a clinically accredited laboratory at the Victorian Clinical Genetics Services (Melbourne, Australia), using a WGS technique. WGS was performed using massively parallel sequencing (MPS) (Nextera DNA Flex Library Prep kit (Illumina Sequencers, San Diego, CA, USA) with a mean target coverage of 30x, and a minimum of 90% of bases sequenced to at least 10x. Sequencing data was generated using Illumina NovoSeq 6000 instruments using S2 flow cells and a 1x150bp paired-end run configuration. Data were processed, including read alignment to the reference genome (GRCh38) and variant calling, using Cpipe (Sadedin et al. 2015) or a functionally equivalent analysis with the Illumina Dragen System (Illumina). Variant analysis and interpretation within the selected target region (RefSeq genes +/- 1kb) was performed using Alissa Interpret (Agilent). Variants were annotated against all RefSeq gene transcripts and reported following HGVS nomenclature.(den Dunnen et al. 2016) Copy number variants (CNV) were screened for using an internal CNV detection tool.(Sadedin et al. 2018) Curation of variants was phenotype-driven with a pre-curated custom gene list for variant prioritization. Variant classification was based on modified ACMG/AMP guidelines in line with ClinGen Sequence Variant Interpretation working group.(Richards et al. 2015; Harrison et al. 2019) Consistent with our clinical accreditation, variants that meet minimum quality criteria for clinical reporting are not in general validated using orthogonal methods.

Urine Metabolic Screen

Urine organic acids were determined by standard gas chromatography-mass spectrometry methodology. Comprehensive targeted urine metabolite screening was performed by flow injection tandem mass spectrometry as previously described (Pitt et al. 2002). Urine thiosulfate was determined by the addition of a -113>-80 *m/z* transition to the above platform.

Newborn Screening

NBS for amino acids and acyl carnitines was performed by standard flow injection tandem mass spectrometry after butylation.

ADDITIONAL INFORMATION

Data Deposition and Access – variant submitted to Clinvar, accession: SCV002016238.1

Ethics Statement – The patients family provided written consent for publication of deidentified patient information, results, pedigree, clinical photography and videography.

Author Contributions – all authors have contributed to patient care and publication and approved the current version of the manuscript and its submission to *CSH Molecular Case Studies*

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FIGURES

(see next pages)

FIGURES

Figure 1. Presenting features: cutaneous manifestations

Clinical photographs taken on Day 4 of admission demonstrating widespread petechiae and purpura to the face, upper-limbs and lower-limbs and torso (not shown)

Figure 2. Presenting features: MRI brain findings

MRI Day 2 post admission: (A) ADC map and (B) corresponding diffusion weighted image (DWI) show multiple punctate lesions in the thalami and globi pallidi that are associated with true diffusion restriction consistent with small acute (likely < 7 days' old) infarcts (arrows). (C) susceptibility-weighted image (SWI) showing signal loss in a few of the right thalamic lesions consistent with microhaemorrhage (arrow). (D) T2 weighted image demonstrating numerous T2 hyperintense periventricular punctate lesions that have no corresponding restricted diffusion (E) consistent with non-acute infarcts. (F) T2 weighted axial image demonstrating subtle widening of the left frontoparietal subarachnoid space (arrows) likely due to reduced left hemispheric volume consequent upon microscopic remote ischaemic white matter/subplate injury postnatally or prenatally.

Figure 3. Pedigree

Figure 4. Presenting features: positional acrocyanosis

Photographs taken by the family in the weeks prior to presentation showing positional acrocyanosis

Supplemental Video 1 – Clonus

Video demonstrating sustained clonus in the proband prior to presentation at 9 months of age

Figure 5. Retrospective re-analysis of newborn bloodspot screening (NBS)

C5 carnitine vs C4 carnitine expressed as multiples of median (log:log scale). P1 is the proband, P2 is an unrelated neonate with a confirmed *ETHE1* defect, red dot is the unaffected sibling of P1, blue dots represent control neonates (n=3215), yellow lines indicate decision limits for a repeat sample (~99.95 percentile).

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