

Neonatal Encephalopathy Associated with a Homozygous ACAD9 Variant of Mitochondrial Complex I Deficiency: A Case Report

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ABSTRACT

Acyl-CoA dehydrogenase 9 (ACAD9)-related mitochondrial disease is an unusual, autosomal recessive disorder with impaired function of complex I of the mitochondrial respiratory-chain and can manifest as severe neonatal encephalopathy. The authors report a male neonate that was born at 37+1 weeks, following elective caesarean section due to previous caesarean delivery and polyhydramnios. At birth, he had poor respiratory effort, generalised hypotonia and apnoea requiring mechanical ventilation. Consanguinity and unexplained multiple neonatal deaths in the family raised a suspicion for an inherited metabolic disorder. The baby went on to have persistent hyperlactataemia, hyperammonaemia, recurrent apnoea and refractory seizures. Infectious investigations were negative and tandem mass spectrometry did not indicate the presence of classical urea-cycle disorders or organic acidemia. Lactate level in the Cerebrospinal Fluid (CSF) was high (more than 15 mg/dL). Brain Magnetic Resonance Imaging (MRI) showed diffusion restriction in the left frontal and parietal lobes, caudate nucleus and centrum semiovale in a non Arterial distribution, which were consistent with metabolic stroke-like lesions. Magnetic Resonance Spectroscopy (MRS) was supportive of Metabolic Brain Injury (MBI). Whole exome sequencing revealed a homozygous ACAD9 c.793A>G (p.Ile265Val) variant which was identified as a variant of uncertain significance. Treatment consisted of riboflavin, thiamine, pyridoxine, L-carnitine, coenzyme Q10, ammonia scavenging and antiepileptic therapy. Lactate and ammonia improved somewhat but neurological deterioration persisted, characterised by refractory seizures and progressive encephalopathy. The baby became very sick with multiorgan dysfunction and succumbed to death during 5th week of life. The lesson to be learned from the present case is that mitochondrial respiratory-chain disorders should be a component of the differential diagnosis in neonates who have unexplained encephalopathy, lactic acidosis, hyperammonaemia and metabolic stroke-like lesions, especially when consanguinity and recurrent unexplained neonatal deaths are present. It also highlights the need to consider segregation and functional studies in the interpretation of variants of unknown significance in ACAD9.

Keywords: Acyl-CoA dehydrogenase 9, Hyperammonaemia, Hyperlactataemia, Mitochondrial encephalopathies, Oxidative phosphorylation, Whole exome sequencing

CASE REPORT

A male infant was born at 37+1 weeks of gestation by elective lower-segment caesarean section for a history of previous caesarean sections [Table/Fig-1]. Birth weight was 2.5 kg. The 27-year-old mother was gravida 6, with three previous live births and two pregnancy losses. None of the previously born infants survived. She was married for 11 years and was in a second-degree consanguineous marriage.

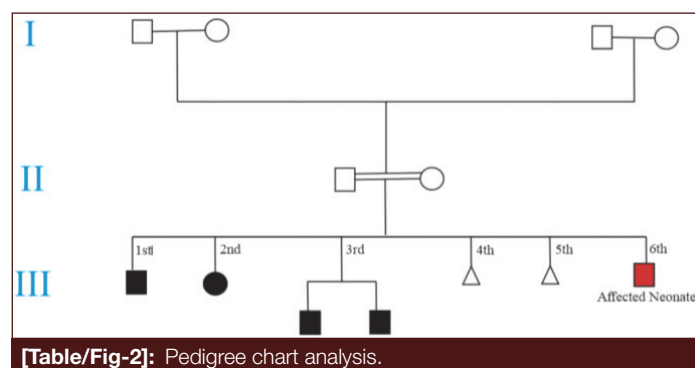


[Table/Fig-1]: Clinical photograph of the neonate.

The pregnancy was spontaneously conceived and was followed with serial antenatal ultrasonography. First-trimester screening showed a singleton live intrauterine gestation with a nuchal translucency of 2.6 mm, presence of the nasal bone and negative screening for trisomies. The anomaly scan at 20+6 weeks showed an estimated foetal weight of 349 g with increased uteroplacental resistance; no structural foetal anomaly was reported. A growth scan at 28 weeks showed appropriate growth, normal Doppler parameters and normal amniotic fluid. At 32 weeks, foetal growth remained appropriate, although increased uteroplacental resistance was noted. The final scan at 35+6 weeks showed a transverse lie, polyhydramnios and small-for-gestational-age status, with an estimated foetal weight of 2.2 kg (7th centile), abdominal circumference at the 28th centile, maintained growth velocity and normal Doppler parameters. Dexamethasone was administered at 34+4 weeks.

The previous obstetric history was significant for two singleton male infants and one Monochorionic Diamniotic (MCDA) twin pregnancy. The first male infant, born in 2014, had intrauterine growth restriction and was diagnosed with Pierre Robin sequence after birth; he died at 10 months of age. The second male infant, born in 2018, died at 12 hours of life. In 2019, the mother had an MCDA twin pregnancy

at 32 weeks, resulting in two male infants weighing 1.9 kg and 2.1 kg. Twin A died at 27 days of life due to sepsis, while Twin B died at 29 days due to respiratory failure. The mother subsequently had a spontaneous abortion at 6+3 weeks and a missed abortion at 7+2 weeks. A genetic evaluation had never been done. The pedigree chart analysis is depicted in [Table/Fig-2].



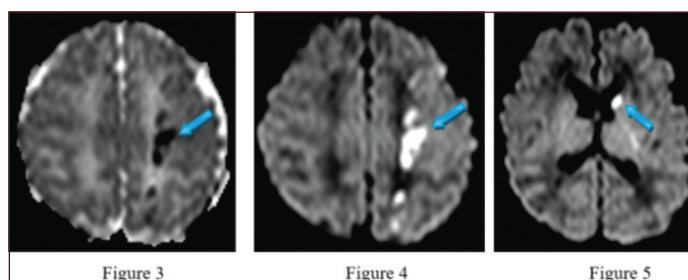
The infant was born with a poor respiratory effort, generalised hypotonia, and apnoea that required bag and mask ventilation and mechanical ventilation. Some encephalopathy symptoms were present with poor reflexes in the neonatal period and requiring ventilator support. Baby had poor sucking, was lethargic. Apnoeic episodes were also present. Pulseoximetry showed tachycardic episodes indicating neonatal seizure. No significant signs of perinatal asphyxia were noted, and blood and Cerebrospinal Fluid (CSF) cultures were sterile. With a negative infectious work-up, consanguinity, bad obstetric history, an inborn error of metabolism was considered.

Differential diagnosis included were hypoxic-ischaemic encephalopathy, neonatal sepsis, urea cycle disorder, organic acidemia, mitochondrial respiratory chain disorders and structural brain abnormalities. A mitochondrial disorder was suspected by the persistence of hyperlactataemia, hyperammonaemia, recurrent apnoea, seizures, consanguinity in the parents and recurrent neonatal deaths.

Metabolic studies showed that hyperlactataemia (peak 35.6 mg/dL) and hyperammonaemia (peak 507.3 $\mu\text{mol/L}$, which reduced to 162 $\mu\text{mol/L}$ while on treatment) were present. There was no abnormality in routine haematological, renal or hepatic investigations. Lactate in CSF was significantly elevated (>15 mg/dL) which indicates dysfunction of cerebral oxidative metabolism. Plasma lactate was not available simultaneously and thus CSF/plasma lactate ratio was not calculated. CSF culture was sterile. There were no abnormalities found by tandem mass spectrometry characteristic of organic acidemia or classical urea cycle disorders.

The clinical course was extremely rapid. On day 1 encephalopathy was present and by day 3 hyperammonaemia and hyperlactataemia were confirmed and high dose riboflavin, mitochondrial cocktail and sodium benzoate was given on day 4. Baby developed non convulsive status epilepticus, manifested by episodes of tachycardia and desaturation for which phenobarbitone was loaded on day 7. Levetiracetam was also added because of recurrent seizure episodes. During the second week, subtle seizure episodes characterised by boxing and cycling movements associated with tachycardia and desaturation were observed. Paediatric neurological consultation was obtained, and lacosamide and phenytoin were subsequently added. No further seizure episodes were documented after day 20, and maintenance phenytoin and levetiracetam were continued.

MRI brain obtained on day 19 revealed diffusion restriction in the left frontal and parietal lobes, left head of caudate nucleus and left centrum semiovale, associated with Fluid-Attenuated Inversion Recovery (FLAIR) hyperintensities, suggestive of metabolic stroke like lesions in a non arterial distribution [Table/Fig-3-5]. MBI was suggested by MRS which demonstrated preserved choline and N-acetyl aspartate peaks, excluding hypoxic-ischaemic insult.



[Table/Fig-3-5]: Hyperintensities in caudate nucleus, left frontal and parietal lobes (blue arrows) in MRI (DWI sequence), suggestive of non arterial pattern of infarction.

Due to the strong family history, consanguinity and metabolic abnormalities, a whole exome sequence was undertaken which identified a homozygous ACAD9 missense variant (c.793A>G; p.Ile265Val). In clinical phenotype, this variant of uncertain significance was extremely similar to the mitochondrial complex I deficiency associated with the ACAD9 gene. There was no segregation analysis performed with the parent and this is a limitation.

Management comprised prolonged mechanical ventilation, seizure control with phenobarbitone, phenytoin, levetiracetam and midazolam infusion, ammonia scavenging with sodium benzoate and sufficient caloric supplementation. On day 4, a mitochondrial cocktail consisting of the vitamins riboflavin (100 mg/day), thiamine (300 mg/day), pyridoxine (50 mg/day), L-carnitine (50 mg/kg/day), and coenzyme Q10 (10 mg/kg/day) was initiated.

Biochemical recovery was partial (serum ammonia and serum lactate), but not neurological, and the infant failed to improve after treatment and continued to decline with refractory seizures and progressive encephalopathy. However, the effect of riboflavin cannot be established, because it is used in combination with several metabolic therapies. Similarly, it cannot be inferred that the clinical course would have been influenced by starting therapy with riboflavin earlier.

In spite of maximal supportive care, infant developed persistent metabolic instability, with multiorgan dysfunction and died on day 35 of life. The family was counselled on the risk of recurrence and molecular testing of the parents with prenatal diagnosis was recommended in future pregnancies.

DISCUSSION

The autosomal recessive disorder, mitochondrial disease, linked to ACAD9 is rare and characterised by a defect in the assembly of the oxidative phosphorylation complex I of the mitochondrial respiratory-chain. The dual function of ACAD9 in both mitochondrial fatty-acid oxidation and complex I assembly is likely to account for the variable clinical presentations of energy demanding organs like the brain, heart and skeletal muscle.

Similar severe neonatal presentations have been reported in patients with ACAD9-related mitochondrial complex I deficiency. Dubucs C et al., described severe antenatal ACAD9 deficiency presenting with foetal growth restriction, cardiomegaly and severe neonatal metabolic disease, demonstrating that ACAD9 deficiency

can manifest during the perinatal period [1]. More recently, Van Hove JLK et al., reported an infant with severe ACAD9 deficiency, lactic acidosis and cardiomyopathy who showed temporary clinical and biochemical improvement with bezafibrate and nicotinamide riboside after an inadequate response to riboflavin. These reports demonstrate the marked phenotypic variability of ACAD9 deficiency, ranging from antenatal or neonatal disease to later-onset neurological and muscular manifestations. In comparison, the present case is distinctive for its predominant early neonatal encephalopathy, hyperammonaemia, recurrent apnoea, refractory seizures and metabolic stroke-like brain lesions, without significant cardiomyopathy [2]. Sinsheimer A et al., showed that the ACAD9 knockout in mice leads to an impairment of mitochondrial function and that tissue specific knockout results in a severe neonatal cardiomyopathy and muscle weakness [3].

In the present neonate, a severe early onset phenotype of generalised hypotonia, apnoea, persistent hyperlactataemia, hyperammonaemia, refractory seizures and progressive encephalopathy resulted in death by day 35. Parental consanguinity and a number of unexplained neonatal deaths and recurrent pregnancy losses indicated that the problem was likely to be an inherited metabolic disorder. Negative infectious investigations and a lack of usual organic acidemia and classical urea cycle disorder findings also favoured a consideration of a mitochondrial respiratory chain disorder. The disease resulting from ACAD9 is a highly phenotypically variable disorder. Dubucs C et al., reported ACAD9-related complex I deficiency, with severe disease occurring in-utero, associated with hypertrophic cardiomyopathy [1].

The high lactate showed the marked elevation of lactate with CSF lactate of more than 15 mg/dL, indicating impaired oxidative metabolism in the brain. Although hyperammonaemia is not a typical biochemical feature of most mitochondrial respiratory-chain disorders, it can occur in selected mitochondrial diseases. In a neonate, the combination of hyperammonaemia with lactic acidosis, recurrent apnoea and progressive neurological deterioration should therefore prompt consideration of an underlying metabolic or mitochondrial disorder, particularly after common urea-cycle and organic acid disorders have been excluded. One of the limitations of this case is that there was no simultaneous plasma lactate measurement to allow the calculation of CSF/plasma lactate ratio [4,5].

Brain MRI showed diffusion restricted to the left frontal and parietal lobes, the caudate nucleus and the centrum semiovale in a non arterial distribution. Such non arterial stroke-like lesions are connected to the metabolic stroke seen in mitochondrial diseases and may superficially look like hypoxic-ischaemic or vascular injuries. Stroke-like lesion as described by Finsterer J can be a helpful clue as lesions often cross vascular distribution and the use of multimodal MRI and MRS can help in differentiation between purely mitochondrial lesions and those that are more typical cerebral infarction [6]. Wang R et al., using proton MRS demonstrated that metabolic abnormalities such as elevated lactate and modified N-acetyl aspartate levels are seen in acute mitochondrial stroke-like lesions [7]. The imaging of this case alongside the other findings including the clinical and biochemical evidence, lead to a diagnosis of MBI and not the more common hypoxic-ischaemic encephalopathy.

Whole-exome sequencing revealed a c.793A>G (p.Ile265Val) homozygous ACAD9 missense mutation. The homozygous state and the consanguinity of the parents and a positive family history were consistent with an autosomal-recessive mode of inheritance. Based on the significant genetic heterogeneity of the mitochondrial diseases, whole-exome sequencing is becoming a valuable tool

in the diagnosis of suspected cases. Sun C et al., showed the ability of exome sequencing in diagnosing patients with suspected mitochondrial disease, and Senaratne TN and Saitta SC highlighted the growing importance of the genomic test in the neonatal population with unexplained clinical features [8,9].

In the present case, however, the identified alteration was a Variant of Uncertain Significance (VUS). Thus, the molecular finding alone is not sufficient in determining the definite pathogenicity. This was incomplete in that no segregation analysis of the parents was done and no functional assay of the activity of complex I was performed. The clinical phenotype is however consistent with ACAD9 disease. The genetic variability and the wide spectrum of clinical diseases that can be caused by variants in ACAD9 have been recently illustrated by Giguet-Valard AG et al., [10]. This underlines the necessity to consider both clinical, biochemical and functional data in combination with the genetic data.

Treatment consisted of riboflavin, thiamine, pyridoxine, L-carnitine, coenzyme Q10, ammonia scavenging, and aggressive anti-seizure therapy. ACAD9 deficiency is especially important with regard to the role of riboflavin, as ACAD9 itself is a flavoprotein, but there are variable responses to treatment. High dose bezafibrate and nicotinamide riboside did acutely reduce lactic acidosis and cardiomyopathy, but resulted in fatal cardiac failure in the infant with severe ACAD9 deficiency who did not respond to riboflavin, demonstrating the currently limited capacity to treat the disease [11].

In the present case, some biochemical improvement was achieved, as there was a decrease in serum ammonia and lactate, but neurological deterioration persisted with refractory seizures and progressive encephalopathy. The therapeutic effect of the riboflavin could not be separated from that of other metabolic therapies given with it. Mancuso M et al., highlighted that seizures can be a symptom of primary mitochondrial disease, can be challenging to manage and can be a poor prognostic indicator [11].

The extensive family history has important implications for genetic counselling. When there are several deaths in the neonatal period, consanguinity and recurrent pregnancy losses, genetic testing should be initiated early in the next pregnancy. A family segregation study and (preferably) functional studies would increase the certainty of the diagnosis, as well as the possibility of carrier testing and targeted prenatal diagnosis of the familial ACAD9 variant.

CONCLUSION(S)

The present case illustrates a very severe neonatal encephalopathy secondary to a homozygous ACAD9 variant, with features of lactic acidosis, hyperammonaemia, metabolic stroke-like brain lesions, refractory seizures and progressive encephalopathy that was fatal. Neonates with unexplained encephalopathy and persistent metabolic abnormalities may have ACAD9-related mitochondrial disease, especially if there are consanguinity and unexplained neonatal deaths in the family. As the identified variant is one that has not been investigated by segregation or functional studies however, it is interpreted with caution regarding its pathogenic role.

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