Long-Term Follow-up for Patients with Mitochondrial Carbonic Anhydrase-5A Deficiency

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Introduction

• Carbonic anhydrase 5A gene (CA 5A, OMIM# 114761) is expressed in the liver, kidneys and skeletal muscles.

• CA 5A encodes mitochondrial carbonic anhydrase VA (CAVA) that catalyses the reversible hydration of CO2 yielding a proton and HCO3⁻ that is required for subsequent biosynthetic processes including ureagenesis, gluconeogenesis and lipogenesis.

• Four enzymes (Figure 1) require the intramitochondrial, CAVA-mediated bicarbonate production including:
  ➢ Carbamoylphosphate synthetase 1 (CPS1).
  ➢ Pyruvate carboxylase (PC).
  ➢ Propionyl CoA carboxylase (PCC).
  ➢ 3-methylcrotonyl CoA carboxylase (3MCC).

![Figure 1: The metabolic pathways affected by CA 5A deficiency: Four enzymes are dependant on the HCO3 produced by CA-VA (coloured in red): CPS1, and three other biotin-dependent carboxylases; PC, PCC and 3MCC.](image-url)
The cases: Two siblings (the index, case 1: II-1, and her brother case 2: II-2) born to healthy nonconsanguineous parents from Belgian-Scottish/English ancestry. Family history: non-contributory.

- **Age of onset:** neonatal period (day of Life (DOL) 2).
- **Presentation:** feeding difficulties, repeated vomiting, tachypnea, irritability, increasing lethargy, and weight loss 9% & 7.7% of birth weight respectively.

**Management:**
- **Acute:** Intravenous Dextrose 10% infusion.
- **Normal Saline (NS) bolus with bicarbonate infusion in the index.
- **Enteral N-carglumic acid 100mg/kg.**
- +/- intravenous lipids 1-2 g/kg/d

**Management:**
- **Chronic:** high-carbohydrate, fat-rich sick-day formula used during intercurrent illnesses.
- **Coenzyme Q 10, Vitamin C supplement.**
- **Supplements:** biotin and thiamine supplements → discontinued at 3 years of age.
- **Levocarnitine 100mg/kg/day → discontinued at the age of 10 & 8 years).**
- **Diet:** regular mixed with no protein restriction.

**Perinatal History:** Both cases were born at term following uneventful pregnancy and delivery. Breast feeding was initiated after the delivery. Birth anthropometric measurement: appropriate for gestational age.

**Initial workup:**
- **Hypoglycemia:** 2.2 & 2.9 mmol/L
- **Hyperammonemia:** 438 & 238 μmol/L
- **Elevated lactate:** 9.8 & 8.8 mmol/L
- **Elevated ketones (8mmol/L ) & large ketonuria.**
- **Respiratory alkalosis with decreased bicarbonate:** (pH: 7.47, pCO2: 10.8 mmHg, and HCO₃⁻: 10.4 mEq/L) & (pH: 7.46, pCO2: 21 mmHg, HCO₃⁻: 21 mEq/L).
- **Creatinine phosphokinase:** elevated in case 1 (1414 U/L), normal in case 2.

**Long term follow up:**
- **Episodes of metabolic decompensation:** Case 1: had three subsequent episodes during concurrent illnesses at 2.5, 3.5 and 8 years requiring hospitalisation for presentation similar to her neonatal presentation and responding to a single dose of enteral N-carglumic acid. **Case 2:** had no additional episodes despite concurrent illnesses.
- **Growth and development:** Case 1 is 13 years and case 2 is 11 years. Both display age-appropriate growth. Formal developmental assessment is pending.
Table 1: Summary of the biochemical, radiological and genetic investigations in cases 1 and 2:

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Case 1</th>
<th>Case 2</th>
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<tbody>
<tr>
<td></td>
<td>DOL 2:</td>
<td>Unremarkable</td>
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<td><strong>Acylcarnitine profile (μmol/L)</strong></td>
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<td><strong>Urine organic acids (mmol/mol creatinine)</strong></td>
<td>DOL 2: Substantial elevation of lactate in urine, marked ketonuria, mild elevation in branched chain ketone and hydroxy acids. Orotic acid was non detectable.</td>
<td>DOL 2: Moderate lactic aciduria and ketonuria, significant increase in pyruvic acid, mild elevation of Krebb cycle intermediates and tyrosine metabolites and dicarboxylic aciduria. Orotic acid and uracil are within reference range.</td>
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<td><strong>Brain imaging (MRI/MRS)</strong></td>
<td>Unremarkable</td>
<td>Petechial focus of diffusion restriction adjacent to the posterior aspect of the trigone of the right lateral ventricle in keeping with small focus of nonspecific white matter injury. MRS: small lactate peak.</td>
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<td><strong>Cardiac evaluation</strong></td>
<td>Echocardiography: both cases had localised area of septal thickness of unknown significance. Repeat: normal cardiac structure and function. ECG: normal sinus rhythm in both cases.</td>
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<td><strong>Additional investigations</strong></td>
<td>Urine amino acids: unremarkable.</td>
<td>Lactate: pyruvate ratio: significantly elevated.</td>
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<td>Chromosomal microarray: normal female.</td>
<td>PC activity in fibroblast: unremarkable.</td>
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<td>PC and TMEM70 gene sequencing: unremarkable.</td>
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<td>Chromosomal microarray: normal male.</td>
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<td><strong>Molecular testing</strong></td>
<td>WES through TIDEX study: Both cases were homozygous for pathogenic variant (c.697T&gt;C, p.Ser233Pro) in CA 5A gene</td>
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CPS1: carbamoylphosphate synthetase 1, ECG: electrocardiograph, NAGS: N-acetylglutamate synthase, MRS: magnetic resonance spectroscopy, PC: pyruvate carboxylase, WES: whole exome sequencing.
Discussion

• CAVA deficiency (OMIM# 615751) is a recessively-inherited inborn error of metabolism that presents with neonatal, infantile or early childhood hyperammonemic encephalopathy, hypoglycemia, hyperlactatemia, hyperketonemia, metabolic acidosis, and metabolic profile suggestive of multiple carboxylase deficiency indicating a defects in the four enzymes dependant on bicarbonate production. Thus, CAVA deficiency should be considered in cases with neonatal hyperammonemia +/- additional biochemical abnormalities.

• Although the clinical presentation may mimic pyruvate carboxylase deficiency, CAVA deficiency can be differentiated by the high excretion of citric acid cycle metabolites in urine organic acids as well as the low-normal citrulline level in plasma amino acids.

• The biochemical profile can be nonspecific, and thus molecular testing by gene panels or WES is recommended.

• Although our cases presented with moderate neonatal presentation, they both display normal growth and development suggesting favourable prognosis.

• In case 2, three subsequent episodes have been reported, all of which improved upon administration of single dose of N-carglumic acid, which has similarly been successfully used in other cases reported in the literature.

• Sick-day formula high in fat and carbohydrate is recommended during concurrent illnesses.

• Early diagnosis and management allows for avoidance of severe complications, and thus decreasing the morbidity and mortality.

Conclusion

CA-5A deficiency is a readily treatable condition. Thus early diagnosis, using combination of biochemical and genetic investigations, is recommended. The long-term follow up is suggested to be favourable, and growth and development remained normal.
Acknowledgement
We would like to thank the patients and their family for their contribution in this case report. Our appreciation is also extended to all clinical and laboratory medical staff in both our centre and in the involved diagnostic laboratories for their participation.

References