

## **Brain Biomarkers of Long-term Outcome of Neonatal Onset Urea Cycle Disorder**

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## Abstract

Urea cycle disorders (UCDs) are common inborn errors of metabolism, with an incidence of one in 30,000 births. They are caused by deficiencies in any of six enzymes and two carrier proteins, the most common being Ornithine Transcarbamylase Deficiency (OTCD). OTCD results in impairment to excrete nitrogen, causing toxic buildup of ammonia with resultant encephalopathy. Hyperammonemia (HA) induces the conversion of glutamate to glutamine in the brain. Excess glutamine in the brain causes osmotic changes cerebral edema, changes in astrocyte morphology, and cell death. Acute symptoms of HA include vomiting, hyperventilation, seizures, and irritability. Long-term neurological changes include deficits in working memory and executive function.

To date, there are no predictors of prognosis of infants with neonatal onset OTCD outside of plasma ammonia level at presentation and duration of hyperammonemic coma. We provide a comprehensive analysis of a 16-year-old male with neonatal onset of OTCD as an example of how brain biomarkers may be useful to monitor disease course and outcome.

This male presented at 8 days post natal with plasma ammonia and glutamine of 677 and 4024 micromol/L and had a missense mutation in Exon 4 (p.R129H). Treatment included protein restriction, sodium benzoate, and citrulline, arginine, and iron. He suffered recurrent acute hyperammonemic episodes despite compliance, triggered by infections or catabolic stressors. We discuss the long-term effects of the hyperammonemic episodes by following MRI based disease biomarkers.

## Introduction

The urea cycle disorders (UCDs) represent a group of rare inborn errors of metabolism characterized by a defect in the metabolism of waste nitrogen from the breakdown of protein and other nitrogen-containing molecules (Figure 1) [1,2]. Severe deficiency or total absence of activity of any of the first four enzymes in the urea cycle (Carbamoyl phosphate Synthetase 1, CPS1; Ornithine transcarbamylase, OTC; Argininosuccinate Synthetase, ASS; and Argininosuccinate Lyase, ASL) or the cofactor producer (N-Acetyl glutamate Synthase, NAGS) results in the accumulation of ammonia and other precursors. Infants with severe UCDs appear normal at birth but quickly develop cerebral edema with resultant lethargy, anorexia, hyper- or hypoventilation, hypothermia, seizures, and coma. In milder (or partial) deficiencies of these enzymes and in arginase (ARG) deficiency, ammonia accumulation may be transient and triggered by illness or stressors.

Proximal UCDs lead to accumulation of ammonia and glutamine (gln), toxic products of protein metabolism [3]. The symptoms of these disorders may present at any age and consequences are neurological of varying severity. Neonatal proximal UCD are usually associated with severe disabilities including encephalopathy, seizures and coma. Without proper treatment, many will succumb to the illness. Increased awareness and institution of early therapy has resulted in less mortality and outcomes, but cognitive sequelae persist [4]. The outcome in childhood onset disease ranges from very mild to severe mental and behavioral sequelae [5]. An emerging group of UCD patients are adolescents and adults with psychiatric manifestations revealing inborn errors of metabolism in adolescents and adults [6].

A common theme of proximal UCDs is hyperammonemia (HA). The majority of patients have substantial cognitive and motor deficits due to HA episodes that may be clinical or subclinical. In the distal disorders, cognitive outcomes may be due to additional brain toxins. Acute and chronic changes in behavior and level of consciousness are reflected in the findings of seizures, encephalopathy, brain edema, and coma.

The pathophysiology by which HA leads to brain injury in UCDs is only partially understood. Episodes of HA cause injury to the brain's white matter. We and others have shown that even "asymptomatic" OTCD is associated with altered neurocognitive profile in an array of cognitive subdomains based in the prefrontal cortex (PFC), such as working memory, executive cognition and attention [7,8,9]. These deficits contribute significantly to disability.

Neuroimaging can help us probe markers of neurological dysfunction in inborn errors of metabolism (IEMs). Magnetic resonance imaging (MRI) approximates microscopic anatomic damage that precedes clinical symptoms [10]. Functional MRI (fMRI) and white matter tractography help one understand how the brain constructs neural networks to perform cognitive tasks and how these networks are altered in brain disorders and recovery [7-9,11,12].

Magnetic resonance spectroscopy (MRS) can allow quantitative sampling of regional, focal, and global changes in metabolism with regard to preclinical changes and response to therapies [13]. Recognition of a number of in vivo neuroimaging techniques, which can reliably and noninvasively assess aspects of neuroanatomy, chemistry, physiology, and pathology, hold promise as biomarkers.

**Patient and methods:** We conducted a retrospective chart review of a 16 year old male patient who presented with neonatal onset of OTCD at University Children's Hospital in Zurich, Switzerland. The patient signed ethics consent to participate in the UCDC studies. The patient had undergone repeated MRI and MR spectroscopy on a 1.5 Tesla Siemens scanner and blood analyses over the course of several years with closer monitoring in infancy period. We abstracted the following data: Plasma glutamine and ammonia levels when available, 1H MRS of white matter and basal ganglia at several key time intervals, routine MRI Images, hospitalization records and neuropsychological testing results.

#### Presentation of the case:

The patient is the second child of healthy, non-consanguineous Swiss parents. The older sister is healthy as well. Diagnosis of OTC deficiency was made after neonatal onset HA with typical laboratory signs including elevated plasma glutamine and urinary orotic acid. The patient required numerous hospitalizations during infancy to treat symptomatic HA. During early childhood, the metabolic situation was more stable but the patient was still hospitalized few times a year. In later childhood and before and during puberty, the situation worsened rendering frequent hospitalizations necessary (Figures 2, 3). Finally, the patient was successfully liver transplanted at age 15 years. Details of the years before liver transplantation were published elsewhere [14].

Metabolic crisis were preceded in most cases by typical triggers including viral infections but there were also several episodes in which no clear trigger was identified. The majority of the

crises could be managed by standard medical treatment with infusions of high glucose, and boluses of nitrogen scavenger drugs (sodium benzoate and/or sodium phenylacetate) followed by continuous infusions of the same drugs. Rarely, the clinical situation required monitoring of the patient on the intensive care unit although several of the episodes were accompanied by severe encephalopathy. There was no need for repeated hemodialysis outside the neonatal period. During long-term management, the patient received a strict protein-restricted diet allowing only intake of the minimal required amount of protein necessary for normal growth. In addition, essential amino acids, trace elements and vitamins were given. As well, the patient needed all available drugs including L-arginine and L-citrulline, and sodium benzoate and sodium phenylbutyrate, which were all given at the maximum recommended dosages. Despite this very challenging treatment, compliance was excellent. The patient underwent brain imaging with MRI/MRS during and after some of the HA episodes and as well almost 2 years after liver transplantation (Figure 4).

The neurological development of the patient was compromised by the recurrent HA crises as well as by the many days he spent in hospital. Formal developmental testing revealed a global DQ score of 73 and 74 at ages 9 and 14 years, respectively (Table 1), and he required transfer to a special school for children with disabilities.

#### Neuroimaging collection:

Imaging was performed on a Siemens 1.5 T Trio system which is a whole body system.  $^1\text{H}$  MRS was used to generate a metabolite profile using single voxel approaches. All measurements were acquired using a manufacturer supplied phased array head coil. For single-voxel spectroscopy a

volume localized PRESS sequence with echo time (TE) of 30 ms, repetition time (TR) of 2000 ms, with a voxel of 2.0 cm on edge (8 ml) centered at the various points of interest was used.

## Results

Several observations are noteworthy and have been observed by others. Plasma glutamine and ammonia levels were not always correlated with one another and glutamine increase may precede the development of HA. Likewise, return to normal plasma ammonia may not be associated with normalization of the plasma ammonia. In this patient, plasma glutamine and brain glutamine levels (measured by  $^1\text{H}$  MRS) both remained elevated during the HA episode even after plasma ammonia levels return to normal following a hyperammonemic episode. Decreased N-acetylaspartate (NAA) peak on MR spectroscopy was observed following hyperammonemic episode. This has not been seen in all patients, especially those with late onset OTCD [15] and suggests neuronal loss or dysfunction. On routine T1 and T2 MRI, there is a relatively normal appearing brain without signal alterations despite elevated glutamine levels, pointing to the utility of  $^1\text{H}$  MRS in the acute and chronic stages of recovery

## Conclusions

This study represents the first comprehensive long-term analysis of a patient with neonatal onset of OTCD. The mechanism leading to ammonia-induced neuropathology in UCD remains uncertain. Clinical signs of hyperammonemia can occur in some patients even at concentrations  $> 60 \mu\text{mol/L}$  and may include anorexia, irritability, lethargy, somnolence, disorientation or other mental status changes. Accumulations of ammonia, Gln, and Glu have been shown to exert toxic effects upon the brain. In animal models, the HA state leads to excitotoxic cell death and, with

prolonged exposure, to the loss of NMDA receptors. These same receptors are altered in the sparse fur (Spf) mouse model of OTCD [16].

The postulated effects of elevated ammonia and Gln include astrocytic swelling [17], an increase in blood brain barrier permeability, disruption of energy through depletion of intermediaries of metabolism including altered amino acid and neurotransmitter levels [18-20]. A rise in plasma Gln levels has also been found to precede HA. Additional evidence for this hypothesis is the presence of elevated brain Gln as measured by  $^1\text{H}$  MRS in patients with OTCD with HA encephalopathy [15]. The results support the view that the encephalopathy associated with HA is related to the elevated concentration of brain Gln.

Gln accumulation is considered neurotoxic and has been implicated in the neuropathology of OTCD. Previous studies in UCD have involved small case series using clinical CT/MRI. Survivors of prolonged HA coma were shown to sustain severe brain pathology including, ventriculomegaly and cortical atrophy. Patients with milder deficits showed bilateral, a/symmetrical low density white matter lesions that were found to be reversible with treatment. The brain biochemical findings resemble those in hepatic encephalopathy. Previous  $^1\text{H}$  MRS studies in patients with hepatic encephalopathy revealed a triad of findings including choline depletion, mI depletion and increased Gln [21-23].

Recently non-interventional variables of disease severity, such as age at disease onset and peak ammonium level of the initial hyperammonemic crisis (cut-off level: 500  $\mu\text{mol/L}$ ) best predicted the neurological outcome [24, 25].



Elevated levels of plasma and brain glutamine despite normal plasma ammonia levels following HA episodes suggest that glutamine may be a better indicator of neurotoxicity. Furthermore, because plasma glutamine and ammonia were not always related, plasma ammonia cannot serve as a reliable clinical marker for neuronal damage. Elevated glutamine and decreased NAA on MR spectroscopy, indicating possible neuronal apoptosis, support this finding. Normal MRI results despite neurocognitive changes, impaired growth, and delayed development suggests that routine imaging may not include the optimal imaging technique to monitor neuronal damage. Our findings have implications for clinical practice and dietary management to prevent cognitive sequelae of hyperammonemia or its effects. Based on this fact and findings on  $^1\text{H}$  MR spectroscopy, we posture that  $^1\text{H}$  MRS should be considered as part of the routine clinical work up in OTCD patients to monitor acute and long-term changes. Further research is needed to prospectively examine the relationship between adverse events and neurocognitive function in OTCD patients.

#### Figure Legends

*Figure 1:* urea cycle enzymes

*Figure 2:* This figure shows the number of Hyperammonemic events that occurred in the patient each year. This figure shows the distribution of the 147 HA episodes over a 15 year span, with many more events in the teen age years.

*Figure 3:* Sequential ammonia and glutamine levels obtained through daily monitoring over a 19-day period. The most striking observation is the persistent elevation of glutamine despite normal ammonia levels.

*Figure 4:* Voxel locations include left parietal white matter on 10/3/08 (a) and 11/18/08 (c) and left basal ganglia on 10/3/08 (b) and 11/18/08 (d) following HA episodes. Glutamine/Glutamate (yellow), NAA (blue), myoinositol, choline (purple), and creatine peak heights are shown. Elevated glutamine peaks are apparent in Figs. 4b, 4c, and 4d. Fig. 4a depicts mildly elevated lactate (gray) and decreased myoinositol peaks. Decreased myoinositol (white) and choline peaks can be seen in Fig. 4c. A notably decreased NAA peak in the basal ganglia is shown in Fig. 4d. Fig 4e. Axial MR image on 11/18/08 through the basal ganglia demonstrating normal brain signal on T2-weighted image. Mild striatal atrophy is present with volume loss involving the caudate and lentiform nuclei.

Table : Cognitive testing results show decreases in working memory and analysis speed from age 9 years to age 14 years.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. Maha Mourad, Johannes Häberle, and Andrea Gropman conceived and designed the study; Johannes Häberle and Tamar Stricker cared for the patient and collected the data; Matthew Whitehead, Andrea Gropman, and Maha Mourad analyzed the data; Maha Mourad, Johannes Häberle, and Andrea Gropman wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest

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