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Brain imaging in Urea cycle disorders

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Abstract

Urea Cycle Disorders (UCD) represent a group of rare inborn errors of metabolism that carry a high risk of mortality and neurological morbidity resulting from the effects of accumulation of ammonia and other biochemical intermediates. These disorders result from single gene defects involved in the detoxification pathway of ammonia to urea. UCD include deficiencies in any of the six enzymes and two membrane transporters involved in urea biosynthesis. It has previously been reported that approximately half of infants who present with hyperammonemic coma in the newborn period die of cerebral edema; and those who survive 3 days or more of coma invariably have intellectual disability [1]. In children with partial defects there is an association between the number and severity of recurrent hyperammonemic (HA) episodes (i.e. with or without coma) and subsequent cognitive and neurologic deficits [2]. However, the effects of milder or subclinical HA episodes on the brain are largely unknown. This review discusses the results of neuroimaging studies performed as part of the NIH funded Rare Diseases Clinical Research Center in Urea Cycle Disorders and focuses on biomarkers of brain injury in ornithine transcarbamylase deficiency (OTCD). We used anatomic imaging, functional magnetic resonance imaging (fMRI), diffusion tensor imaging (DTI), and $^1\text{H}/^{13}\text{C}$ magnetic resonance spectroscopy (MRS) to study clinically stable adults with partial OTCD. This allowed us to determine alterations in brain biochemistry associated with changes in cell volume and osmolarity and permitted us to identify brain biomarkers of HA. We found that white matter tracts underlying specific pathways involved in working memory and executive function are altered in subjects with OTCD (as measured by DTI), including those heterozygous women who were previously considered asymptomatic. An understanding of the pathogenesis of brain injury in UCD is likely to advance our knowledge of more common disorders of liver dysfunction.

Keywords

biomarkers; hyperammonemia; neuroimaging; urea cycle; MRI; fMRI; MRS; DTI

Although several theories exist, it is not well understood how hyperammonemia (HA) disrupts brain function. In addition, the pathogenesis of brain injury and recovery from neurological sequelae associated with UCDs remains largely unexplored. The time course of metabolic perturbation in the brain is also unknown and has not been previously studied. As

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part of our NIH- funded Rare Diseases Clinical Research Center in Urea Cycle Disorders, we are investigating these questions by using advanced magnetic resonance imaging methods that allow real time assessment of brain metabolic perturbations and biomarkers in UCD. Our work focuses on a defined population of UCD patients, those with ornithine transcarbamylase deficiency (OTCD), the only X-linked urea cycle disorder.

Clinicians who care for affected patients commonly encounter clinical settings characterized by significant elevations of plasma concentrations of ammonia and glutamine without neurological dysfunction, as well as situations in which patients manifest confusion, vomiting and ataxia in the presence of only mild elevations of blood ammonia and glutamine. Surrogate brain markers that can be used clinically to predict severity of insult and potential treatment response will impact management decisions and the development of neuroprotective strategies that can be used within a critical time window. However, prediction of outcome is not straightforward. There is currently no direct correlation between genotype, peak ammonia level, structural changes in the brain and/or phenotype [3, 4]. The age of onset, duration and degree of HA are used to establish the prognosis and the extent to which the neurological changes may be reversible, but the predictive value is limited [2, 5-9].

The majority of patients with neonatal onset OTCD do have substantial cognitive and motor deficits resulting from hyperammonemic episodes [10, 11]. Males with partial deficiencies, manifest somewhat less severe cognitive, motor and psychiatric symptom [12]. Approximately 85% of heterozygous females are believed to be asymptomatic based on their clinical history, whereas the remainder has behavioral and learning disabilities, protein intolerance, cyclical vomiting, stroke-like episodes and hyperammonemic coma [13-19]. Evidence of specific neurocognitive deficits in these patients have been reported [20]. Despite average IQ scores, they display a specific neurobehavioral phenotype characterized by a nonverbal learning disability, associated with white matter or subcortical dysfunction. We have shown recently that neuroimaging with ¹H MRS, DTI, and fMRI can uncover abnormalities in the brains of subjects with partial OTCD, reflecting cellular injury in otherwise normal appearing brain by conventional MRI [21]. This has important implications for understanding the mechanism of brain injury in this disorder and other UCDs. In addition, these findings provide brain specific biomarkers for clinical monitoring and intervention studies. These studies form the substance of this paper.

Brain Pathology in UCD

It has been established that HA coma in neonatal onset OTCD is associated with severe brain injury. The mechanisms leading to injury include glutamine (Gln) induced cerebral edema, mitochondrial energy failure and alterations in neurotransmitters[6]. Ammonia can diffuse freely or be transported across the blood-brain barrier in amounts proportional to arterial blood concentration and blood flow. Metabolic trapping occurs in the form of Gln, which can reach concentrations in the brain that are higher than in peripheral blood [22, 23]. Ammonia entering the brain is converted to Gln by glutamine synthetase (GS). This enzyme offers short-term buffering of excess ammonia, but Gln is osmotically active and can cause astrocytic swelling, leading to cytotoxic edema [24-26]. The GS inhibitor methionine sulfoximine has been found to protect against ammonia-induced brain edema in both *in vivo* and *in vitro* animal models [27]. Gln is the main nitrogen scavenger of the brain, and its formation within the astrocyte from ammonia and glutamate is part of the Gln/Glu cycle which is perturbed during HA (Figure 1).

Neonatal onset OTCD shares similarities to hepatic encephalopathy and hypoxic ischemic encephalopathy (HIE) in that the degree of damage is proportional to the duration of

hyperammonemia and interval between coma and death [28]. Neuropathology in neonatal onset OTCD is manifest by cortical atrophy, ventriculomegaly, hypomyelination or myelination delay, and cystic changes of white matter [29-31]. In late onset OTCD, reversible white matter lesions are observed.

Neuroimaging studies performed months after acute neonatal HA coma correlate with pathological findings, demonstrating hypomyelination of white matter, myelination delay, cystic changes of the white matter and, with prolonged HA, gliosis of the deep gray matter nuclei [32-34]. Accumulations of ammonia, Gln, and Glu (the principal excitatory neurotransmitter) have been shown to exert toxic effects on the brain. In animal models, the hyperammonemic state produces excitotoxic cell death and, with prolonged exposure, the loss of N-methyl-d-aspartate (NMDA) receptors [35].

Water imbalance

Ammonia can cause brain damage by overloading the ability of glial cells to make Gln, resulting in brain swelling. Aquaporins (AQPs) are a family of water selective channels. Brain AQPs play important roles in the regulation of water homeostasis and cerebrospinal fluid formation [36]. Recently, AQP4 and AQP9 have been reported to be involved in the brain water accumulation that occurs in brain edema [37]. Studies of transgenic mouse and brain injury models have found that AQP4 may play a role not only in the edema formation but also in fluid elimination [37]. Recent data from expression profiling of astrocytes isolated acutely from hyperammonemic mouse brains suggest disturbed water and potassium homeostasis as a potential mechanism of brain edema associated with HA coma [38].

Neurocognitive deficits in OTCD

Children rescued from neonatal HA coma have shown a significant risk for the development of neurocognitive sequelae [2, 4, 10, 11, 39] However, whether these deficits are manifest in mild or “asymptomatic” individuals carrying a mutation for OTCD is largely unexplored. Such information would have impact on clinical management and dietary guidelines. Using standardized measures, Gyato et al. evaluated neuropsychological performance in 19 women heterozygous for OTCD. Cognitive performance was compared with clinical status (symptomatic vs. asymptomatic), genotype (neonatal type vs. late onset mutation), and urea synthetic capacity measured by [¹⁵N] ammonia incorporation into urea. This study provided evidence for specific neurocognitive deficits in patients with partial OTCD. Deficits were characterized by a nonverbal learning disability, which is typically associated with white matter or subcortical dysfunction. These findings demonstrated that women with OTCD who were previously considered asymptomatic harbor subtle yet significant neurocognitive deficits that correlate with a known underlying pathology in white matter [20]. Despite average IQ scores, these subjects displayed a specific neurobehavioral phenotype. Weaknesses were identified in nonverbal intelligence, fine motor/dexterity/speed, visual memory, attention and executive skills, and math.

Based on these results, we sought to validate a neurocognitive measure of executive function and working memory that could be used in conjunction with neuroimaging and intervention studies. We found that two validated neurocognitive tests were sensitive in picking up brain alterations in our OTCD cohort: the color STROOP and the Trails part B (Figure 2). The STROOP test assesses simple attention, gross reading speed and divided attentional abilities [40]. It is also a measure of executive functioning, requiring the participant to inhibit an over learned response in favor of an unusual one. Participants are instructed to read a series of words that are presented in varying colors. Each word is a color – red, blue, green, etc. For example, participants see the word “red,” which would be easy to read if it was printed in

red. But, in this task it is printed in blue. Participants must read the word itself, ignoring the color the word is printed in. The task requires keen attentional skills and is useful in assessing participants' speed of processing abilities. The Comprehensive Trail Making Test part B consists of five trials of visual search and sequencing tasks that are heavily influenced by attention, concentration, resistance to distraction, and cognitive flexibility. This test is a nonspecific marker of brain injury [41].

Use of neuroimaging to study brain alterations in UCD

Neuroimaging can provide information about the timing, extent, reversibility and possible mechanism of neural injury in a noninvasive manner and can be used as an adjunctive measure to predict clinical and neurocognitive outcome. We have used a number of neuroimaging platforms to assess the effects of metabolic disorders on the brain including functional MRI (fMRI, to study integrity of neural networks), diffusion tensor imaging (DTI, to measure microscopic white matter integrity), and proton magnetic resonance spectroscopy (¹H MRS, to provide biochemical information and allow a noninvasive measure of brain metabolism under steady state and dynamic conditions) [42].

Routine T1 and T2 images can be used to characterize gray matter and white matter microstructural and macrostructural changes. When abnormal brain pathology exists, there may be signal abnormalities in T1 and T2 weighted scans. The limitations of routine imaging are that one is only able to detect damage at a macroscopic level, typically when the patient is already symptomatic; MRI findings may also lag behind clinical changes. In disorders where there is white matter damage, the use of FLAIR imaging has added the ability to detect early white matter injury and understand the full extent of macroscopic white matter injury [43] (Figure 3).

To further evaluate the earliest measures of disruption of white matter integrity, we used diffusion-tensor imaging (DTI), a MR imaging technique in which contrast is based on differences in the diffusion of water molecules [44]. DTI is used to identify and generate maps of white matter fibers. Diffusion-tensor MRI relates image intensities to the relative mobility of water molecules in tissue and allows for inference of direction of the motion. The motion of water molecules is a random walk (Brownian motion). Anisotropy refers to the property of being directionally dependent. Cellular diffusion of water corresponds to cell geometry in axons, and therefore, diffusion MRI can also be used to make inferences about white matter architecture. Three major indices of diffusion in tissue can be quantitated: magnitude (ADC), predominant orientation of diffusion, and degree of anisotropy (orientation of diffusion and deviation from uniform diffusion in all directions). The more unrestricted the water molecules, the higher the ADC and the lower the anisotropy. DTI has been used to make predictions in recovery from brain injury [45].

fMRI is a modality of imaging used to map changes in brain hemodynamics that correspond to cognitive operations [46]. fMRI provides high resolution, non-invasive reports of neural activity detected by a blood oxygen level dependent signal. It allows one to directly observe brain function, brain organization and recovery after injury. It can provide useful information about neurological status and risk. The signal in fMRI is derived from increased blood flow to the local vasculature that accompanies neural activity in the brain. This results in a local reduction in deoxyhemoglobin and an increase in blood flow without an increase in oxygen extraction [46]. Deoxyhemoglobin is paramagnetic and alters the T2* weighted magnetic resonance image signal; this is the source of the signal for fMRI. Due to the ability to image the entire 3-dimensional volume of brain, fMRI is capable of isolating many simultaneous and coordinated brain events and can be used to evaluate executive function, high level cognitive processes, and neural reorganization after injury [47].

Magnetic resonance spectroscopy (MRS) is based on the principle that the MR signal that is detectable from a volume element, or voxel, is directly proportional to the concentration of the nuclei. For example, a concentrated chemical such as water at 55.5 mmol per gram can be detected by MR imaging using voxels from about 1 mm³ to 5 mm³. However, the concentrations of brain biochemicals are lower relative to water, so that special water suppression and shimming must be performed to overcome this limitation. On a routine short scan, one can quantitate the following chemicals: lactate, N-acetyl aspartate (NAA), Gln/Glu, creatine, choline, and myoinositol (mI). Other chemicals may be present in certain pathologies (phenylalanine, valine, succinate, etc.).

¹H MRS studies in OTCD

The mechanism leading to ammonia-induced neuropathology in UCD remains uncertain. Clinical signs of hyperammonemia can occur at concentrations > 60 micromol/L and include anorexia, irritability, lethargy, somnolence, disorientation, vomiting, cerebral edema, and coma. 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 [48].

The postulated effects of elevated ammonia and Gln include astrocytic swelling [25], an increase in blood brain barrier permeability, disruption of energy through depletion of intermediaries of metabolism and altered amino acid and neurotransmitter levels [49-51]. Elevated brain Gln by ¹H MRS has been documented in hepatic encephalopathy [52, 53] as well as in experimentally induced HA. 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 ¹H MRS in patients with OTCD with HA encephalopathy [32, 33]. 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 ¹H MRS studies in patients with hepatic encephalopathy revealed a triad of findings including choline depletion, mI depletion and increased Gln.

We used single voxel ¹H MRS to investigate cerebral metabolism in stable patients with partial OTCD in five brain regions (Figure 4). We quantified metabolite concentrations using independent linear combination modeling (LC Model) [54]. We studied a total of 25 subjects with partial OTCD (males and females) and compared the information with 25 age matched controls. We detected significant increases of Gln levels ($p < 0.007$) in posterior cingulate gray matter (PCGM), parietal white matter (PWM), and frontal white matter (FWM) in subjects with OTCD (symptomatic and asymptomatic subjects) compared to controls.

mI concentrations were significantly decreased in parietal and frontal white matter (PWM, FWM), thalamus and PCGM [21] (Figure 5). The degree of brain mI depletion was inversely correlated with brain Gln level (Figure 6). This inverse relationship between Gln and mI was not observed in controls (Figure 6). Interestingly, reduced level of mI in white matter was observed in women with OTCD who were asymptomatic and suggests the possibility of unrecognized biochemical disturbances (such as edema and volume changes in

the astrocyte) in these subjects. The reduction of mI also correlated with cognitive impairments in a pattern suggesting a white matter injury model. Thus, we believe that low mI is a biomarker of a prior HA episode and represents a compensatory mechanism to correct astrocytic swelling due to high Gln. In addition, choline (Cho) reduction in frontal white matter was observed in our cohort. As Cho-containing compounds relate to membrane turnover, membrane dysfunction preceding neuronal death is suggested to occur in the frontal cortex in OTCD heterozygotes. This dysfunction may be responsible for some of the neurocognitive deficits observed in this disorder. Overall comparison of brain biochemical differences in our cohorts is shown in Figure 7.

We also used ^1H MRS and urea synthetic capability to evaluate brain metabolic differences in two adult sisters heterozygous for OTCD who had discordant clinical courses (symptomatic and asymptomatic). This permitted us to assess whether changes in the brain biochemicals Gln and mI correlated with urea synthetic ability and clinical severity (see pedigree in Figure 8). We performed single voxel 3.0T ^1H MRS in these women during a stable medical state and compared their imaging to an age matched adult control. We also compared them to 13 subjects with partial OTCD and to 12 controls who had been previously studied. All three subjects, as well as the symptomatic OTCD mother of the two sisters, had neurocognitive testing and previous stable isotope studies to measure urea synthetic capacity [55].

We found IQ scores to be inversely correlated with the symptoms in these subjects (i.e. more symptoms, lower IQ score). Findings of decreased mI identified all OTCD subjects, even the sister who was asymptomatic. mI was decreased in FWM, PWM, PCGM, and THA in both the symptomatic and asymptomatic OTCD sisters. The degree of mI decrease was inversely correlated with the disease severity score, a measure reflecting total number and severity of hyperammonemic episodes and IQ scores. Choline was decreased in both OTCD subjects in the frontal white matter, especially in the symptomatic sister (Figure 9). We found that the concentration of brain mI was inversely correlated with the clinical phenotype in these three female members of the family with a known neonatal onset OTCD mutation. We also found that despite the asymptomatic heterozygous having near normal residual urea synthetic capacity, ^1H MRS disclosed a brain metabolic derangement with regard to the mI level [56] (Figure 9).

^{13}C MRS studies in OTCD

While ^1H MRS is a sensitive tool to detect brain biochemical abnormalities in individual patients, the specificity suffers from the complex peak pattern due to J-coupling and signals from different compounds co-resonating at similar chemical shifts. *In vivo* ^{13}C MRS can reliably quantitate distinct signals from Glu and Gln. Unambiguous assignment of these metabolites can contribute to a better understanding of the pathogenesis and treatment of brain dysfunction in UCD and is an innovative approach that will advance knowledge in this field. In contrast to conventional proton MRS, which non-invasively assays metabolites at their steady-state concentrations, ^{13}C MRS depends upon the absence of natural abundance signal in normal brain, and the highly specific chemical shift of MR signal that arises from the stable isotope when introduced via the blood stream into the normal brain metabolite pools.

Glutamate neurotransmission (GNT) is carried out by a glial neuronal process that includes the oxidation of glucose and the Gln/Glu cycle [57]. This allows for direct *in vivo* measurement of GNT using proton-decoupled ^{13}C MRS. The metabolic model predicts that under conditions of elevated plasma ammonia the increase in the rate of Gln synthesis is stoichiometrically coupled to the increase in the uptake of the anaplerotic substrates CO_2

and ammonia and the efflux of Gln from the brain. Studies in hyperammonemic rats suggest that only a fraction of Gln is used to synthesize GABA via Glu. The remainder is passed through the neuronal TCA cycle before being used to synthesize GABA. Bluml et al. have shown that there is disturbed neurotransmitter Glu/Gln cycling in chronic hepatic encephalopathy [58]; Glu enrichment is decreased and Gln enrichment is increased.

^{13}C MRS allows measurement of cerebral glucose uptake and metabolism through the TCA cycle leading to an understanding of cerebral brain metabolism that can be correlated with disease severity in OTCD. The natural abundance of glucose is 1%; therefore enrichment with a nonradioactive ^{13}C labeled substrate administered intravenously is required to follow the fate of label incorporation into Gln, Glu, aspartate, and finally to CO_2 (Figure 10).

During pilot studies performed in collaboration with Dr. Brian Ross of the Huntington Magnetic Resonance Research Institute, we have found preliminary evidence that GNT is altered in adults with OTCD (Figure 10) [59]. We used the occipital lobe voxel location and more recently frontal lobe voxel location (with low power deposition) [60]. Similar observations have been made in patients with hepatic encephalopathy. Moreover, fMRI and neuropsychological testing revealed deficits in working memory and executive function attributable to white matter injury affecting frontal lobe processes.

Most studies using ^{13}C MRS have determined cycling from isotopic labeling of Gln and Glu using a $[1-^{13}\text{C}]$ glucose tracer, which provides label through neuronal and glial pyruvate dehydrogenase or via glial pyruvate carboxylase. To measure the anaplerotic contribution, we studied ^{13}C incorporation into Gln and Glu in the occipital-parietal region of awake patients. We used an infusion of $[2-^{13}\text{C}]$ glucose, which labels the C2 and C3 positions of Gln and Glu exclusively via pyruvate carboxylase. This study suggests that the glial anaplerotic pathway activity is not substantially altered in OTCD patients. Rather we consider that the defect lies in the major metabolic pathways through neurons and glia, which contribute to glutamate neurotransmission in the well-known Gln/Glu cycle. However, the use of $[2-^{13}\text{C}]$ glucose also served another valuable purpose. Because this approach allowed detection of ^{13}C brain metabolism without the forbidden proton-decoupling conventionally employed, we were able to safely examine frontal brain regions to confirm a similar pattern of metabolism.

Diffusion Tensor Imaging Discloses Changes in White Matter in OTCD

To determine if there are white matter microstructural abnormalities in partial OTCD that could underlie the cognitive phenotype, we used diffusion tensor imaging (DTI). We focused on white matter alterations because prior neuropathology studies in patients dying of HA coma showed white matter abnormalities, and there is a known relationship between Gln toxicity and white matter damage. In contrast to the water in CSF which diffuses without restriction, in the highly-structured myelinated white matter, tissue water diffuses in a highly directional, or anisotropic, manner. DTI can thus be used to identify and characterize disruptions in myelination of white matter pathways (as reflected by a more random or disordered tissue water) that are the substrate for functional neural networks. The ability to examine the integrity of these pathways provides a link between anatomical and functional neuroimaging. ADC and anisotropy are believed to be related predominantly to the integrity of axonal tracts.

We hypothesized that changes in white matter microstructure would be found in pathways that correlate with the neurocognitive profile of OTCD. We studied 25 adult patients with partial OTCD and 25 age-matched adult controls. Diffusion indices in regions of interest (ROIs) were quantified and compared between the study group and controls by using the Mann-Whitney rank-sum test. We found significant decreases in fractional anisotropy (FA)

of cingulum white matter indicating changes in white matter microstructure in these regions (Figure 11). Additionally, when correlated with severity scores, there was an inverse relationship between FA and disease severity. As fiber tracts connecting these regions underlie executive function and working memory, DTI provides an objective means for determining the relationship to cognitive deficits in OTCD. The finding of reduced FA in white matter tracts related to frontal lobe function suggests an anatomic correlate to our findings of deficits of executive function in OTCD heterozygotes, even those who are asymptomatic.

fMRI in UCD

fMRI provides detailed maps of brain areas underlying human mental activities. The effect of HA on brain activation during performance of working memory tasks under high memory load has not previously been evaluated in OTCD. We hypothesized that activation maps would differ in subjects with partial OTCD in regions that underlie the cognitive deficits in executive function and working memory. In our cohort of 25 adults with partial OTCD and 25 age matched adult controls using a working memory task, we found the accuracy and speed of performance of this task were equal in subjects and controls. Subjects with OTCD who performed the same memory load as controls, however, demonstrated more diffuse frontal and extra frontal activation. In normal controls, high load conditioning activates a region in the left prefrontal cortex, as well as in the right prefrontal and striatal regions, which activate during low load conditions. Our results combined with results from DTI and MRS suggest impaired frontal lobe processing in subjects with OTCD that is compensated for by recruitment of additional cortical and subcortical areas. We also found a more developmentally immature activation pattern in individuals with OTCD.

Neuroimaging in Arginase Deficiency

We have begun collecting imaging data in other disorders of nitrogen metabolism. Arginase deficiency is the least common of the UCD. This disorder clinically presents differently from other UCD (which present as hyperammonemic encephalopathy), often manifesting as spastic diplegia which may be indistinguishable from cerebral palsy. The typical presentation is that of an older infant with delayed motor development and a history of episodes of vomiting and somnolence without apparent cause. These children often are protein intolerant and show evidence of long-tract neurological impairment. A common clinical feature in this disorder is spasticity, and many affected children are incorrectly diagnosed with cerebral palsy. We used DTI to evaluate the white matter tracts in a patient with arginase deficiency, postulating that the deficit would be distinctly different from that in OTCD and predominantly involve the corticospinal tracts. We compared diffusion tensor imaging data from a 17 year old male with arginase deficiency, age-matched normal controls, and age-matched individuals with OTCD. As predicted, significant differences were found in suspected areas of interest, specifically the corticospinal tracts were affected in arginase deficiency and not OTCD (Figure 12) [61].

Summary of Imaging findings in UCD

This review has discussed the results of neuroimaging studies performed as part of Rare Diseases Clinical Research Center in Urea Cycle Disorders with major focuses on biomarkers of brain injury in adults with partial ornithine transcarbamylase deficiency (OTCD). While routine clinical MRI sequences tend to be normal, use of FLAIR imaging improves recognition of white matter injury. The use of advanced imaging sequences such as fMRI, DTI and spectroscopy (1H and 13 C MRS) provide additional details about the pattern and type of injury. In OTCD, important biochemical brain markers of injury include increased gln levels and depletion of mI, markers of disturbed osmotic balance due to HA

that can be measured quantitatively on ¹H MRS. DTI shows a pattern of white matter injury affecting the cingulum, a major fiber bundle which underlies pathways involving working memory and attention. Patients with late onset OTCD showed altered neural circuitry by fMRI when performing working memory and attentional tasks. In contrast, DTI in Arginase deficiency, a UCD with unique clinical presentation (spastic diplegia) demonstrates predilection of brain injury in corticospinal tracts with additionally decreased fiber density. Preliminary studies with ¹³C MRS found a glutamate neurotransmission deficit in patients with late onset OTCD, implying deficits in glutamate pathways may impair learning and memory.

Future Directions

In continuing the UCD neuroimaging project, we propose to expand our studies to better define the time course of brain biochemistry, osmotic, and white matter biomarkers seen in clinically stable OTCD as well as in those patients recovering from acute HA crises. The ability of the brain to buffer osmotic changes by a decrease in mI concentrations should be predictive of recovery and clinical outcome. These studies will help define the contribution of age, stage of myelination, and early metabolic changes in predicting resolution and subsequent neurocognitive outcome which will impact clinical care. Once these biomarkers are validated, they can be used to assess the stringency of treatments required to optimize outcome and to address the question as to whether patients with even “asymptomatic” OTCD should receive medical management to avoid long term effects on cognition. Validation of brain biomarkers and their correlation with clinical status will allow us to move forward toward their application as endpoints in evaluation of current and novel therapies for UCD that will improve neurological outcome.

Abbreviations

HA	hyperammonemia
MRI	magnetic resonance imaging
UCD	urea cycle disorder
DTI	diffusion tensor imaging
fMRI	functional MRI
MRS	magnetic resonance spectroscopy

References

1. Summar ML, Dobbelaere D, Brusilow S, Lee B. Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes. *Acta Paediatr.* 2008; 97:1420–1425. [PubMed: 18647279]
2. Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED. Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. *N Engl J Med.* 1984; 310:1500–1505. [PubMed: 6717540]
3. Breningstall GN. Neurologic syndromes in hyperammonemic disorders. *Pediatr Neurol.* 1986; 2:253–262. [PubMed: 3334203]
4. Nicolaides P, Liebsch D, Dale N, Leonard J, Surtees R. Neurological outcome of patients with ornithine carbamoyltransferase deficiency. *Arch Dis Child.* 2002; 86:54–56. [PubMed: 11806886]
5. Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. *N Engl J Med.* 2007; 356:2282–2292. [PubMed: 17538087]

6. Bachmann C. Long-term outcome of patients with urea cycle disorders and the question of neonatal screening. *Eur J Pediatr.* 2003; 162(Suppl 1):S29–33. [PubMed: 14634803]
7. Bachmann C. Ornithine carbamoyl transferase deficiency: Findings, models and problems. *J Inherit Metab Dis.* 1992; 15:578–591. [PubMed: 1528018]
8. Picca S, Dionisi-Vici C, Abeni D, Pastore A, Rizzo C, Orzalesi M, Sabetta G, Rizzoni G, Bartuli A. Extracorporeal dialysis in neonatal hyperammonemia: Modalities and prognostic indicators. *Pediatr Nephrol.* 2001; 16:862–867. [PubMed: 11685590]
9. Uchino T, Endo F, Matsuda I. Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. *J Inherit Metab Dis.* 1998; 21(Suppl 1):151–159. [PubMed: 9686352]
10. Msall M, Monahan PS, Chapanis N, Batshaw ML. Cognitive development in children with inborn errors of urea synthesis. *Acta Paediatr Jpn.* 1988; 30:435–441. [PubMed: 3150233]
11. Gropman AL, Batshaw ML. Cognitive outcome in urea cycle disorders. *Mol Genet Metab.* 2004; 81(Suppl 1):S58–62. [PubMed: 15050975]
12. Drogari E, Leonard JV. Late onset ornithine carbamoyl transferase deficiency in males. *Arch Dis Child.* 1988; 63:1363–1367. [PubMed: 3202644]
13. Batshaw ML, Roan Y, Jung AL, Rosenberg LA, Brusilow SW. Cerebral dysfunction in asymptomatic carriers of ornithine transcarbamylase deficiency. *N Engl J Med.* 1980; 302:482–485. [PubMed: 7351973]
14. Christodoulou J, Qureshi IA, McInnes RR, Clarke JT. Ornithine transcarbamylase deficiency presenting with strokelike episodes. *J Pediatr.* 1993; 122:423–425. [PubMed: 8441099]
15. DiMagno EP, Lowe JE, Snodgrass PJ, Jones JD. Ornithine transcarbamylase deficiency--a cause of bizarre behavior in a man. *N Engl J Med.* 1986; 315:744–747. [PubMed: 3748082]
16. Gaspari R, Arcangeli A, Mensi S, Wismayer DS, Tartaglione T, Antuzzi D, Conti G, Proietti R. Late-onset presentation of ornithine transcarbamylase deficiency in a young woman with hyperammonemic coma. *Ann Emerg Med.* 2003; 41:104–109. [PubMed: 12514690]
17. Mizoguchi K, Sukehiro K, Ogata M, Onizuka S, Watanabe J, Yoshida I, Yoshino M. A case of ornithine transcarbamylase deficiency with acute and late onset simulating reye's syndrome in an adult male. *Kurume Med J.* 1990; 37:105–109. [PubMed: 2255172]
18. Perini M, Zarcone D, Corbetta C. Hyperammonemic coma in an adolescent girl: An unusual case of ornithine transcarbamylase deficiency. *Ital J Neurol Sci.* 1993; 14:461–464. [PubMed: 8282523]
19. Rimbaux S, Hommet C, Perrier D, Cottier JP, Legras A, Labarthe F, Lemarcis L, Autret A, Maillot F. Adult onset ornithine transcarbamylase deficiency: An unusual cause of semantic disorders. *J Neurol Neurosurg Psychiatry.* 2004; 75:1073–1075. [PubMed: 15201380]
20. Gyato K, Wray J, Huang ZJ, Yudkoff M, Batshaw ML. Metabolic and neuropsychological phenotype in women heterozygous for ornithine transcarbamylase deficiency. *Ann Neurol.* 2004; 55:80–86. [PubMed: 14705115]
21. Gropman AL, Fricke ST, Seltzer RR, Hailu A, Adeyemo A, Sawyer A, van Meter J, Gaillard WD, McCarter R, Tuchman M, Batshaw M. 1H MRS identifies symptomatic and asymptomatic subjects with partial ornithine transcarbamylase deficiency. *Mol Genet Metab.* 2008; 95:21–30. [PubMed: 18662894]
22. Felipe V, Butterworth RF. Neurobiology of ammonia. *Prog Neurobiol.* 2002; 67:259–279. [PubMed: 12207972]
23. Ott P, Clemmesen O, Larsen FS. Cerebral metabolic disturbances in the brain during acute liver failure: From hyperammonemia to energy failure and proteolysis. *Neurochem Int.* 2005; 47:13–18. [PubMed: 15921824]
24. Willard-Mack CL, Koehler RC, Hirata T, Cork LC, Takahashi H, Traystman RJ, Brusilow SW. Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat. *Neuroscience.* 1996; 71:589–599. [PubMed: 9053810]
25. Norenberg MD, Rao KV, Jayakumar AR. Mechanisms of ammonia-induced astrocyte swelling. *Metab Brain Dis.* 2005; 20:303–318. [PubMed: 16382341]
26. Takahashi H, Koehler RC, Brusilow SW, Traystman RJ. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. *Am J Physiol.* 1991; 261:H825–829. [PubMed: 1679605]

27. Takahashi H, Koehler RC, Brusilow SW, Traystman RJ. Glutamine synthetase inhibition prevents cerebral oedema during hyperammonemia. *Acta Neurochir Suppl (Wien)*. 1990; 51:346–347. [PubMed: 1982483]
28. Batshaw ML, Brusilow S, Waber L, Blom W, Brubakk AM, Burton BK, Cann HM, Kerr D, Mamunes P, Matalon R, Myerberg D, Schafer IA. Treatment of inborn errors of urea synthesis: Activation of alternative pathways of waste nitrogen synthesis and excretion. *N Engl J Med*. 1982; 306:1387–1392. [PubMed: 7078580]
29. Harding BN, Leonard JV, Erdohazi M. Ornithine carbamoyl transferase deficiency: A neuropathological study. *Eur J Pediatr*. 1984; 141:215–220. [PubMed: 6734670]
30. Kornfeld M, Woodfin BM, Papile L, Davis LE, Bernard LR. Neuropathology of ornithine carbamyl transferase deficiency. *Acta Neuropathol*. 1985; 65:261–264. [PubMed: 3976361]
31. Dolman CL, Clasen RA, Dorovini-Zis K. Severe cerebral damage in ornithine transcarbamylase deficiency. *Clin Neuropathol*. 1988; 7:10–15. [PubMed: 3370859]
32. Connelly A, Cross JH, Gadian DG, Hunter JV, Kirkham FJ, Leonard JV. Magnetic resonance spectroscopy shows increased brain glutamine in ornithine carbamoyl transferase deficiency. *Pediatr Res*. 1993; 33:77–81. [PubMed: 8433866]
33. Takanashi J, Barkovich AJ, Cheng SF, Kostiner D, Baker JC, Packman S. Brain mr imaging in acute hyperammonemic encephalopathy arising from late-onset ornithine transcarbamylase deficiency. *AJNR Am J Neuroradiol*. 2003; 24:390–393. [PubMed: 12637287]
34. Takanashi J, Kurihara A, Tomita M, Kanazawa M, Yamamoto S, Morita F, Ikehira H, Tanada S, Kohno Y. Distinctly abnormal brain metabolism in late-onset ornithine transcarbamylase deficiency. *Neurology*. 2002; 59:210–214. [PubMed: 12136059]
35. Robinson MB, Hopkins K, Batshaw ML, McLaughlin BA, Heyes MP, Oster-Granite ML. Evidence of excitotoxicity in the brain of the ornithine carbamoyltransferase deficient sparse fur mouse. *Brain Res Dev Brain Res*. 1995; 90:35–44.
36. Kruse E, Uehlein N, Kaldenhoff R. The aquaporins. *Genome Biol*. 2006; 7:206. [PubMed: 16522221]
37. Yang B, Zador Z, Verkman AS. Glial cell aquaporin-4 overexpression in transgenic mice accelerates cytotoxic brain swelling. *J Biol Chem*. 2008; 283:15280–15286. [PubMed: 18375385]
38. Lichter-Konecki U, Mangin JM, Gordish-Dressman H, Hoffman EP, Gallo V. Gene expression profiling of astrocytes from hyperammonemic mice reveals altered pathways for water and potassium homeostasis in vivo. *Glia*. 2008; 56:365–377. [PubMed: 18186079]
39. Krivitzky L, Babikian T, Lee HS, Thomas NH, Burk-Paull KL, Batshaw ML. Intellectual, adaptive, and behavioral functioning in children with urea cycle disorders. *Pediatr Res*. 2009; 66:96–101. [PubMed: 19287347]
40. King JA, Colla M, Brass M, Heuser I, von Cramon D. Inefficient cognitive control in adult adhd: Evidence from trial-by-trial stroop test and cued task switching performance. *Behav Brain Funct*. 2007; 3:42. [PubMed: 17708762]
41. Mirsky AF. Behavioral and psychophysiological markers of disordered attention. *Environ Health Perspect*. 1987; 74:191–199. [PubMed: 3319553]
42. Gropman AL. Expanding the diagnostic and research toolbox for inborn errors of metabolism: The role of magnetic resonance spectroscopy. *Mol Genet Metab*. 2005; 86:2–9. [PubMed: 16276565]
43. Kim KW, MacFall JR, Payne ME. Classification of white matter lesions on magnetic resonance imaging in elderly persons. *Biol Psychiatry*. 2008; 64:273–280. [PubMed: 18471801]
44. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. *Biophys J*. 1994; 66:259–267. [PubMed: 8130344]
45. Bendlin BB, Ries ML, Lazar M, Alexander AL, Dempsey RJ, Rowley HA, Sherman JE, Johnson SC. Longitudinal changes in patients with traumatic brain injury assessed with diffusion-tensor and volumetric imaging. *Neuroimage*. 2008; 42:503–514. [PubMed: 18556217]
46. Li K, Guo L, Nie J, Li G, Liu T. Review of methods for functional brain connectivity detection using fmri. *Comput Med Imaging Graph*. 2009; 33:131–139. [PubMed: 19111443]
47. Nakamura T, Hillary FG, Biswal BB. Resting network plasticity following brain injury. *PLoS One*. 2010; 4(12):e8220. [PubMed: 20011533]

48. Qureshi IA, Rao KV. Sparse-fur (spf) mouse as a model of hyperammonemia: Alterations in the neurotransmitter systems. *Adv Exp Med Biol.* 1997; 420:143–158. [PubMed: 9286431]
49. Bachmann C, Colombo JP. Increase of tryptophan and 5-hydroxyindole acetic acid in the brain of ornithine carbamoyltransferase deficient sparse-fur mice. *Pediatr Res.* 1984; 18:372–375. [PubMed: 6201810]
50. Butterworth RF. Effects of hyperammonaemia on brain function. *J Inherit Metab Dis.* 1998; 21(Suppl 1):6–20. [PubMed: 9686341]
51. Watanabe A, Shiota T, Takei N, Nagashima H. Excitatory and inhibitory amino acid neurotransmitters and ammonia metabolism in hepatic failure rats. *Res Exp Med (Berl).* 1985; 185:399–404. [PubMed: 2865779]
52. Kreis R, Ross BD, Farrow NA, Ackerman Z. Metabolic disorders of the brain in chronic hepatic encephalopathy detected with H-1 MR spectroscopy. *Radiology.* 1992; 182:19–27. [PubMed: 1345760]
53. Ross BD, Jacobson S, Villamil F, Korula J, Kreis R, Ernst T, Shonk T, Moats RA. Subclinical hepatic encephalopathy: Proton MR spectroscopic abnormalities. *Radiology.* 1994; 193:457–463. [PubMed: 7972763]
54. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton nmr spectra. *Magn Reson Med.* 1993; 30:672–679. [PubMed: 8139448]
55. Yudkoff M, Daikhin Y, Nissim I, Jawad A, Wilson J, Batshaw M. In vivo nitrogen metabolism in ornithine transcarbamylase deficiency. *J Clin Invest.* 1996; 98:2167–2173. [PubMed: 8903337]
56. Gropman AL, Seltzer RR, Yudkoff M, Sawyer A, VanMeter J, Fricke ST. 1H MRS allows brain phenotype differentiation in sisters with late onset ornithine transcarbamylase deficiency (OTCD) and discordant clinical presentations. *Mol Genet Metab.* 2008; 94:52–60. [PubMed: 18262815]
57. Westergaard N, Sonnewald U, Schousboe A. Metabolic trafficking between neurons and astrocytes: the glutamate/glutamine cycle revisited. *Dev Neurosci.* 1995; 17:203–211. [PubMed: 8575339]
58. Blüml S, Moreno-Torres A, Ross BD. [1-13C] glucose MRS in chronic hepatic encephalopathy in man. *Magn Reson Med.* 2001; 45:981–993. [PubMed: 11378875]
59. Gropman AL, Sailasuta N, Harris KC, Abulseoud O, Ross BD. Ornithine transcarbamylase deficiency with persistent abnormality in cerebral glutamate metabolism in adults. *Radiology.* 2009; 252:833–841. [PubMed: 19567648]
60. Sailasuta N, Robertson LW, Harris KC, Gropman AL, Allen PS, Ross BD. Clinical NOE 13C MRS for neuropsychiatric disorders of the frontal lobe. *J Magn Reson.* 2008; 195:219–225. [PubMed: 18829354]
61. Oldham MS, VanMeter JW, Shattuck KF, Cederbaum SD, Gropman AL. Diffusion tensor imaging in arginase deficiency reveals damage to corticospinal tracts. *Pediatr Neurol.* 2010; 42:49–52. [PubMed: 20004862]

Figure 1.

The relationship between the neuron and astrocyte glutamine handling. Glutamate in the synapse is taken up by the neuron, shuttled to the astrocytes and used to synthesize glutamine which then diffuses back to the neuron and is converted to glutamate. Glutamate, an excitatory neurotransmitter is used to make GABA, the major CNS inhibitory neurotransmitter.

Figure 2. STROOP and Comprehensive trials making test, part B show differences in subjects with OTCD and controls. The subjects are further separated into those who are symptomatic and asymptomatic.

Figure 3.

White matter lesions seen in patients with OTCD. In the top panel are routine T2 weighted scans where the recognition of T2 hyperintensities is not obvious. In the bottom panel, after applying FLAIR imaging, the areas of T2 signal hyperintensity become more apparent.

Figure 4.

Voxel locations chosen for ^1H MRS; a) parietal white matter; b) posterior cingulate gray matter; c) thalamus; d) frontal white matter; e) frontal gray matter. (reprinted from Gropman AL, Fricke ST, Seltzer RR, Hailu A, et al (2008). *Mol Genet Metab.* 95(1-2):21-30).

Figure 5.

Biochemical areas of significance in OTCD by brain region (reprinted from Gropman AL, Fricke ST, Seltzer RR, Hailu A, et al (2008). *Mol Genet Metab.* 95(1-2):21-30). These bar graphs demonstrate the concentrations of the major measured metabolites in brain in subjects and controls. Regions of significance between subjects and controls are shown as a p-value.

Figure 6.

There is an inverse relationship between brain glutamine and myoinositol levels in subjects with OTCD as measured by 1H MRS. No such relationship was observed in the controls. (reprinted from Gropman AL, Fricke ST, Seltzer RR, Hailu A, et al (2008). *Mol Genet Metab.* 95(1-2):21-30)

Figure 7.

This overlapping spectrum acquired by ^1H MRS shows the major brain metabolite differences in subjects with OTCD and controls. The Black indicates an OTCD subject and the gray an age matched control subject (reprinted from Gropman AL, Fricke ST, Seltzer RR, Hailu A, et al (2008). *Mol Genet Metab.* 95(1-2):21-30).

Figure 8.

Pedigree of OTCD family. The two probands are III-1 and III-3. Proband III-1 is asymptomatic and III-3 is symptomatic and presented at age 2.5 years with anorexia, vomiting and encephalopathy. Their mother II-2 is mildly symptomatic. There is a history of neonatal onset disease with lethality in a male. Case II.1 is an OTCD heterozygote who is clinically asymptomatic. Her sister, case III.3 presented at age 2-1/2 with anorexia, vomiting and encephalopathy. She experiences intermittent hyperammonemia and cognitive impairments. Their mother, case II.2, had vomiting and headache with onset of teen years (reprinted from Gropman AL, Fricke ST, Seltzer RR, Hailu A, et al (2008) *Mol Genet Metab.* 95(1-2):21-30).

Figure 9.

This overlapping spectrum shows contributions from 1H MRS: An overlay of the two sisters and normal control is seen (Gropman AL, Seltzer RR, Yudkoff M, Sawyer A, et al (2008) *Mol Genet Metab.* 94(1):52-60).

Figure 10.

¹³C-labeled substrates can be infused to enhance the MR signal obtained from a localized brain region. The high specificity of the ¹³C spectrum can be exploited, and the time course of labeled metabolites (shown in filled and partially filled circle) can be tracked through important metabolic pathways (figure compliments of Dr. Brian Ross, Huntington Medical Research Institute, Pasadena, CA).

Figure 11.
Cingulum fibers are highlighted as having a decreased FA (see text).

Figure 12. Significant difference in FA between case subject (arginase deficiency) and normal controls in the pons.