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Chapter

Emerging Knowledge From Noninvasive Imaging Studies: Is Ammonia Control Enough?

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Abstract

Multiple lines of research suggest that ammonia is harmful to the brain if the levels remain elevated for extended periods of time. Several decades ago, there was no testing or standard of care to monitor the effect of hyperammonemia (HA) on neurological function in urea cycle disorders (UCD), and the timing of HA encephalopathy is still not clear. Magnetic resonance imaging (MRI) was not done routinely, if at all, so it was not known what changes were occurring in the brain, during and after recovery from HA. Decades ago, a diagnosis of a UCD meant severe disability and early death. Earlier diagnosis, improved management, and nitrogen scavenger therapy have improved the lives and life span of patients with UCD. However, many patients suffer from learning difficulties under the umbrella "executive function" which comprises neurologically based skills involving mental control and self-regulation. The general agreement of the core elements of executive functions includes inhibition, working memory, and cognitive flexibility and is necessary in development of skills in reasoning, fluid intelligence, problem-solving, and planning. Our research focuses on the use of noninvasive neuroimaging coupled with neuropsychological testing to understand the complex relationship between ammonia, glutamine, cognitive function, seizures, and specifically impact on development of working memory.

Keywords: ammonia, EEG, glutamine, MRI, neuroimaging, urea cycle disorder

1. Introduction

The urea cycle disorders (UCD) represent one of the most common groups of inborn errors of metabolism, with an overall incidence of 1 in 30,000 [1, 2], and involve deficiency of one of six urea cycle enzymes or of a related cofactor or transporter [3, 4]. The most common of these, ornithine transcarbamylase deficiency (OTCD), is the only disorder of ureagenesis inherited in an X-linked manner, with an estimated incidence of 1 in 70,000 [5]. Over 240 missense mutations have been identified in the OTCD gene but overall 400 including nonsense, frameshift, in-frame indels, splice site errors, and one in a regulatory domain [6, 7]. About 60% of hemizygous males harbor a mutation around the enzyme active site and present with hyperammonemic (HA) coma in the newborn period [8, 9]. The remaining 40% of patients demonstrate more peripheral mutations in other parts of the gene, associated with less severe phenotypes and later onset presentation [10].

A majority of children with OTCD have cognitive and motor deficits due to hyperammonemic episodes [8, 11–13]. Neonatal onset disease mortality rate is high. Prior to advances in recognition and treatment, it was not uncommon for survivors of neonatal onset disease to have intellectual disability, cerebral palsy, and seizures [14]. Neonatal survivors have a decreased IQ which may be as low as 43. In males with partial deficiencies, disease onset is later, and outcome is better, although still associated with high mortality and morbidity with many individuals manifesting cognitive, motor, and psychiatric sequelae [15–17], in particular impaired working memory and other measures of executive function which are essential for performing well in school, vocations, and relationships. Treatment of OTCD involves a combination of protein restriction (which is also a restriction in nitrogen, leading to ammonia accumulation) and medications that invoke an alternative pathway of waste nitrogen excretion [18, 19]. Females heterozygous for OTCD have a variable phenotype and display a broad range of symptoms from apparently asymptomatic to fully affected, owing to both allelic heterogeneity and differential X-inactivation patterns.

A common presumption for years has been that approximately 85% of heterozygous females are asymptomatic based on history, whereas the remainder show symptoms ranging from behavioral and learning disabilities and protein intolerance to cyclical vomiting, stroke-like episodes, and hyperammonemic coma [20–23]. Symptomatic women who harbor mutations seen in the neonatal onset disorder in hemizygous males [10] may develop HA due to skewed X-inactivation. There is therefore a range of residual enzyme capacities and urea synthetic capacities that result in this variation [24]. However, advances in neuroimaging and the work of the Urea Cycle Disorders Consortium (UCDC) have demonstrated that many of these previously presumed asymptomatic females have similar brain structural, biochemical, and cognitive biomarkers seen in those who are clinically impacted, yet they may be mild under conditions of low demand. More obvious symptoms were uncovered when cognitive demand increases or there is superimposed illness or stressor [25].

2. Pathophysiology of the UCDs

Ammonia is a product of the metabolism of proteins and other compounds, and it is required for the synthesis of essential cellular compounds. However, a five- to tenfold increase in ammonia in the blood induces toxic effects in most animal species, with alterations in the function of the central nervous system. Ammonia is a normal constituent of all body fluids. At physiologic pH, it exists mainly as ammonium ion. Reference serum levels are less than 35 mmol/L (outside the newborn period, where higher levels are seen). Excess ammonia is excreted as urea, which is synthesized in the liver through the urea cycle. Sources of ammonia include bacterial hydrolysis of urea and other nitrogenous compounds in the intestine, the purine-nucleotide cycle and amino acid transamination in skeletal muscle, and other metabolic processes in the kidneys and liver. Increased entry of ammonia to the brain is a primary cause of neurological disorders associated with HA, such as congenital deficiencies of urea cycle enzymes, hepatic encephalopathies, Reye syndrome, several other metabolic disorders, and some toxic encephalopathies [26–28].

On the basis of studies in animal models and other preclinical model systems, several mechanisms of ammonia neurotoxicity at the molecular level have been proposed.

While the exact pathophysiology remains unclear, current theories include (1) glutamine accumulation, with associated impaired cerebral osmoregulation, and (2) glutamate/*N*-methyl D-aspartate (NMDA) receptor activation, with resultant excitotoxic injury and energy deficit [26–30]. The WM is preferentially affected in proximal UCD, and the extent of injury has been shown to depend upon the duration of HA coma and the interval between coma and death [28].

Acute ammonia intoxication in an animal model leads to increased extracellular concentration of glutamate in the brain and results in activation of the NMDA receptor. Activation of this receptor mediates ATP depletion and ammonia toxicity; blocking the NMDA receptor with dizocilpine (MK-801) prevents both phenomena. The ATP depletion is due to activation of Na^+/K^+-ATPase, which, in turn, is a consequence of decreased phosphorylation by protein kinase C. Activation of the NMDA receptor may account for seizures seen in some patients during acute HA [29].

High levels of ammonia in the brain also induce other metabolic changes that are not mediated by activation of the NMDA receptor and thus are not involved directly in ammonia-induced ATP depletion or neurotoxicity. These include increases in brain levels of lactate, pyruvate, glutamine, and glucose, with concomitant decreases in brain glycogen, ketone bodies, and glutamate [30].

Chronic HA is associated with an increase in inhibitory neurotransmission as a consequence of two factors. The first involves the downregulation of glutamate receptors secondary to excessive extrasynaptic accumulation of glutamate. The second mechanism implies increased GABAergic tone resulting from benzodiazepine receptor overstimulation by endogenous benzodiazepines and neurosteroids. These changes likely play a role in central nervous system features of intellectual function, decreased consciousness, and coma [26, 30].

In the brain, glutamine represents a storage depot for nitrogen, binding excess ammonia and offering a short-term buffering of excess ammonia in patients with HA, likely as a protective mechanism. It is these high levels of glutamine in the brain that are also hypothesized to be neurotoxic and one of the factors leading to injury (UCDC unpublished). Brain astrocytes are key players in the interactions of glutamine and ammonia via the Gln/Glu cycle (**Figure 1**).

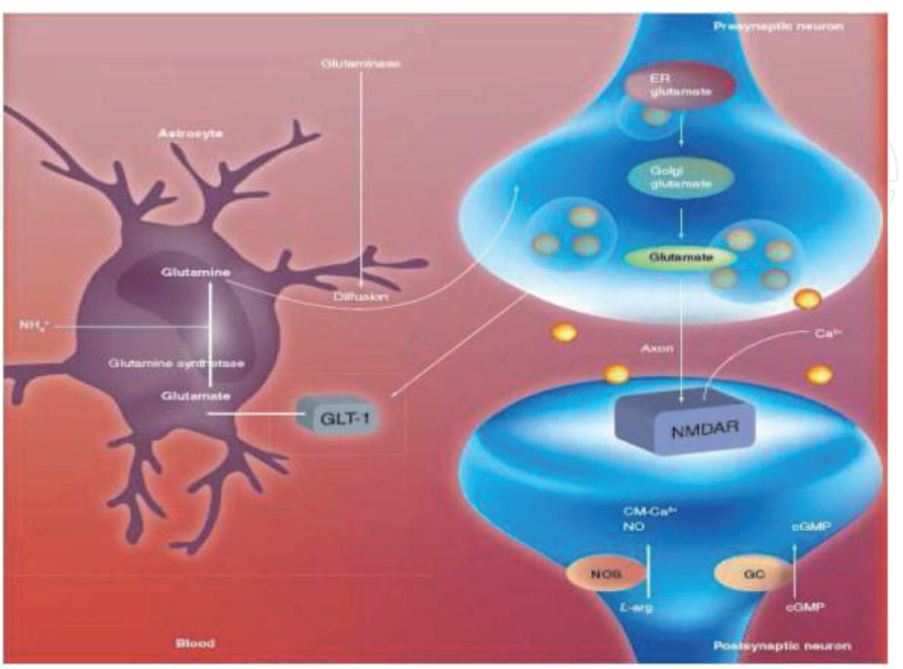

Figure 1.
*The glutamine-glutamate cycle. Reprinted with permission from Pediatric Health, 2008, **2**(6):701–713.*

When ammonia is not adequately detoxified by the hepatic urea cycle, there is an increase in scavenger amino acids, including glutamine. Ammonia entering the brain is rapidly incorporated into the formation of glutamine by glutamine synthetase, present in the astrocyte. Glutamine concentrations increase in hyperammonemic states. While not measured directly, indirect measures with ^{1}H magnetic resonance spectroscopy (MRS) studies of patients with urea cycle disorders have demonstrated elevations of the glutamine/glutamate complex [30]. Glutamine has been implicated in hyperammonemic encephalopathy. It has been shown that a rise in plasma glutamine levels precedes HA. There is a sustained positive correlation between plasma glutamine and ammonia levels.

Inhibition of glutamine synthetase in hyperammonemic rats by treatment with enzyme inhibitors prevents the rise in cortical glutamine levels and cortical water content [31]. Clearance of synaptic glutamate by glial cells is required for the normal function of excitatory synapses and to prevent neurotoxicity. This process occurs in the atrocity, which takes up synaptic glutamate and returns glutamate to the neurons in the form of glutamine, a non-neuroactive amino acid that the neurons subsequently reconvert to glutamate via the action of mitochondrial phosphate-dependent glutaminase.

2.1 Short-term clinical effects of HA

Clinical signs of HA may occur at concentrations >60 micromol/L and are very individual as some patients may tolerate higher levels before symptoms are noticed. The short-term changes may include initially anorexia, irritability, lethargy, somnolence, disorientation, vomiting, and asterixis (flapping tremor). As symptoms progress and ammonia is not lowered, cerebral edema, coma, herniation and death [30]. In the acute stages, there is increased blood brain barrier permeability, leading to depletion of intermediates of cell energy metabolism. On an anatomic level, there is disaggregation of microtubules [29].

2.2 Chronic effects of HA

Chronic effects of HA include alterations in axonal development as well as alterations in brain amino acid and neurotransmitter levels. Electrophysiologic effects of HA include direct effects on inhibitory postsynaptic potentiation (IPSP). Neurotransmission is impacted due to increased extracellular glutamate levels and downregulation of AMPA-kainate receptors, enhanced tryptophan uptake, elevated quinolinic acid levels, and enhanced NMDA activity. Activation of NMDA receptors increases calcium in postsynaptic neurons which binds to calmodulin and activates neuronal nitric oxide (NO) synthase, increasing NO, which activates guanylate cyclase, increasing cyclic guanine monophosphate (cGMP), part of which is released to the extracellular space [31, 32]. Activation of this glutamate- NO-cGMP pathway may be involved in some forms of learning.

Recent reports indicate that guanylate cyclase and cGMP are important in learning and memory; induction of LTP is the molecular basis of some forms of learning and memory [33]. Because glial cells also have these receptors, the excessive glutamate leads to glial cell swelling, which seems to protect the neurons from excitotoxic injury. Studies in spf mouse models of OTC and other animal models of HE show neuropathological evidence of excitotoxic neuronal cell death which suggests that overactivation of NMDAR is a feature of urea cycle disorders [34] and may be age dependent [35].

3. What are the cognitive implications of HA on the brain?

A proportion of individuals with OTCD have a wide spectrum of neuropsychological complications including developmental delay, intellectual disability, and executive function deficits [36]. Most adult-onset patients remain asymptomatic, until they present with rapid decline in mental status and subsequently chronic encephalopathy [36, 37].

Fluctuating HA may cause delirium, confusion, and incoherent speech. In addition to subsequent regression, lack of attention leads to unemployment and introverted behavior [37]. Waisbren et al. demonstrated that nearly all asymptomatic 156 women with OTCD attained a full-scale intelligence quotient (IQ) of 102 ± 16. Among 25 men, the full-scale IQ measured was 101 ± 21. No differences were noted between the verbal and performance scores. In addition, in 27% of females and 33% of males, working memory deficiency was observed as a constant finding. The ammonia concentration and its duration appear to be key determinants of the long-term outcome [37].

3.1 Long-term sequelae of HA: Executive function

Executive function (EF) is the ability to control and regulate actions and thoughts [38]. It includes processes such as working memory, self-regulation, and inhibitory control.

Executive functions are a set of cognitive processes and competencies that control behavior and learning. It is an umbrella term which comprises neurologically based skills involving mental control and self-regulation. The general agreement of the core elements of executive functions includes inhibition, working memory, and cognitive flexibility [38, 39]. These elements are highly interrelated, and the interplay of these processes is vital in flexible, goal-directed behaviors. From the core elements of basic executive functions, higher-order executive functions such as reasoning, fluid intelligence, problem-solving, and planning are built [40].

Historically, executive functioning has been thought to be regulated by the prefrontal cortex of the frontal lobes; however reviews found indications for the sensitivity, but not for the specificity, of executive function measures to the frontal lobe [41]. Both the frontal brain regions and other structures of the brain are involved and necessary for successful application of these skills.

Individuals are not born with executive function skills, but rather are born with the potential to develop them. With any genetic or environmental insult to the brain, the executive functions and prefrontal cortex are one of the first to suffer and suffer disproportionately. The disruption of the brain architecture can seriously delay or impair the development of executive functioning [38].

To date, most evaluations of EF rely on parents' reports such as the Behavior Rating Inventory of Executive Function Preschool (BRIEF-P) form, which may not capture the development of executive skills to its full expression [42].

EF has been previously studied using task-based functional MRI (fMRI) scans, which can be difficult to adapt for children [43]. Instead, multiple studies have relied on resting state functional MRI [44]. Reineberg et al. investigated differences in brain connectivity in relation to individual performances during different EF behavioral tasks [45]. However, to our best knowledge, resting state fMRI has not been applied to characterize EF-related brain connectivity differences in children, especially at a young age (2–5 years old).

4. How can neuroimaging help us probe markers of neurological dysfunction in IEMs?

Multiple studies using multimodal MRI suggest its value in the recognition of microscopic anatomic damage that *precedes clinical symptoms* in many inborn errors of metabolism and neurodegenerative disorders. Depending upon the type of imaging study, it may answer a different question regarding the pathology, biochemistry, or physiology. Neuroimaging may detect subtle abnormalities that can be correlated with neurocognitive abnormalities even in asymptomatic OTCD heterozygotes. The neuroimaging/neurocognitive studies we performed as part of the UCDC focused on adolescents and adults with OTCD. Our collective studies demonstrated that OTCD heterozygous females have changes in function of the prefrontal cortex (PFC) in association with an altered neurocognitive profile in working memory, executive functioning, and attention [46].

fMRI can allow us to understand how the brain constructs neural networks to perform cognitive tasks, probe how these networks are altered in brain disorders, and allow us to follow recovery. Magnetic resonance spectroscopy using hydrogen (^{1}H) or carbon (^{13}C) allows us to probe both static and dynamic changes in brain metabolism [47]. The benefits of neuroimaging using MRI are the ability to view the brain in the three orthogonal views, the lack of radiation exposure, and the ability to target the organ or pathology being studied. In addition, high-performance MR hardware is available resulting in faster scans and higher resolution with higher field.

4.1 Use of neuroimaging to assess brain injury in UCDs

Neuroimaging in recent years has come to encompass many different modalities that can be combined in a single imaging session to gain complementary information regarding the brain's structural, functional, and metabolic dimensions.

A typical routine structural MRI protocol includes not only T1- and T2-weighted sequences but also, in most academic and teaching hospitals, fluid attenuation inversion recovery (FLAIR) and voxel-based morphometry (VBM) or other ability to measure tissue volume from acquired structural images on a clinical scanner. Diffusion weighted and diffusion tensor imaging (DWI and DTI) are used to study microstructural variance in WM fiber tracts [48], and proton magnetic resonance spectroscopy is used to measure brain metabolism in static and dynamic models [47]. Multimodal assessment batteries and data fusion give investigators a complex and varied perspective into the structural, functional, and biochemical parameters of the central nervous system in IEMs [49].

4.2 What MRI modalities are available and what do they measure?

4.2.1 Magnetic resonance imaging (MRI)

MRI interrogates tissue water protons via differential populations of proton spins that result when a biological sample is placed in a strong magnetic field. Using MRI, one can define brain anatomy and characterize gray matter and WM microstructural and macro-structural changes. These are read as signal abnormalities on T1- and T2-weighted images which correspond with the specific tissue pathologies. With MRI one can detect damage at a macroscopic level. One must remember that MRI findings can lag behind clinical changes and stages of disease as well as recovery processes.

4.2.2 Fluid-attenuated inversion recovery

Fluid-attenuated inversion recovery imaging is usually a routine part of most radiology clinical imaging sequences. Diffusion tensor imaging (DTI) is used due to its sensitivity in detecting increases in interstitial water content. Such applications include imaging brain tumors, demyelinating diseases (i.e., multiple sclerosis), metabolic WM disease cerebral infarcts, and gliotic scars.

4.2.3 Diffusion weighted imaging and diffusion tensor imaging

Diffusion MRI is another MRI method which allows fine mapping of the diffusion process of molecules (i.e., water) in biological tissues. DWI and DTI can be used in vivo noninvasively. DWI and DTI techniques are focused on the fact that molecular diffusion in tissues is not free but, rather, is impacted by obstacles, such as macromolecules and cell membranes (myelin). By using DTI, water molecule diffusion patterns can inform about microscopic details regarding myelin integrity and architecture.

DTI relates image intensities to the relative mobility of water molecules in the tissue. It can also imply direction of the motion [48]. In general, areas that have a relatively high mean diffusion appear dark on the diffusion weighted MRI images. Diffusion MRI can also be used to make inferences about WM architecture since the diffusion of water corresponds to cell geometry in axons [48].

Cytotoxic edema in contrast follows sodium/potassium pump failure, often due to energy metabolism failure due to ischemic insult. It is quick and can occur within minutes of the onset of ischemia and can produce increased brain tissue water of up to 3–5%. In HA, cytotoxic edema also results. Therefore, DTI can be used in patients with UCD at baseline to assess whether patients differ with respect to WM integrity. DTI has a role in measuring functional connectivity differences between control groups and patients and to follow up patients over time to monitor disease progression, recovery, or impact of therapies [50]. It is a very good technique to also look at rapid fluxes in water content such as during a HA episode in a patient with UCD and allows follow-up noninvasively during recovery and/or with introduction of a therapeutic agent.

The most commonly used indices for the measurement of anisotropic diffusions by DTI include the relative anisotropy measure, fractional anisotropy (FA), and the volume ratio indices. These indices provide quantitative measurements of the changes of WM integrity in brain regions that are affected by diseases.

We have used DTI techniques together with advanced fiber tracking algorithms, to evaluate the 3D trajectories of neural tracts. This has allowed us to model WM neural connectivity in UCDs. DTI was used to determine whether there are WM microstructural abnormalities in partial OTCD that could underlie the cognitive phenotype. Our focus on WM alterations was based on prior neuropathology studies in HA. These studies have shown WM is almost exclusively affected. There is also a relationship between Gln toxicity and WM damage [51]. Anisotropy was calculated by standard methods, from the eigenvalues of the diffusion tensor by using the FA metric. After comparison between UCD patients and age-matched control groups, we established that FA of the frontal WM was significantly decreased in patients with UCD compared to the age-matched controls. This, in turn, is indicative of changes in WM microstructure (**Figure 2**). Additionally, we found an inverse relationship between FA and disease severity that was not age dependent. Based on this, we could conclude that MR imaging in OTCD may be normal in patients with late-onset disease, heterozygotes, or those not in hyperammonemic crisis at the time of the study.

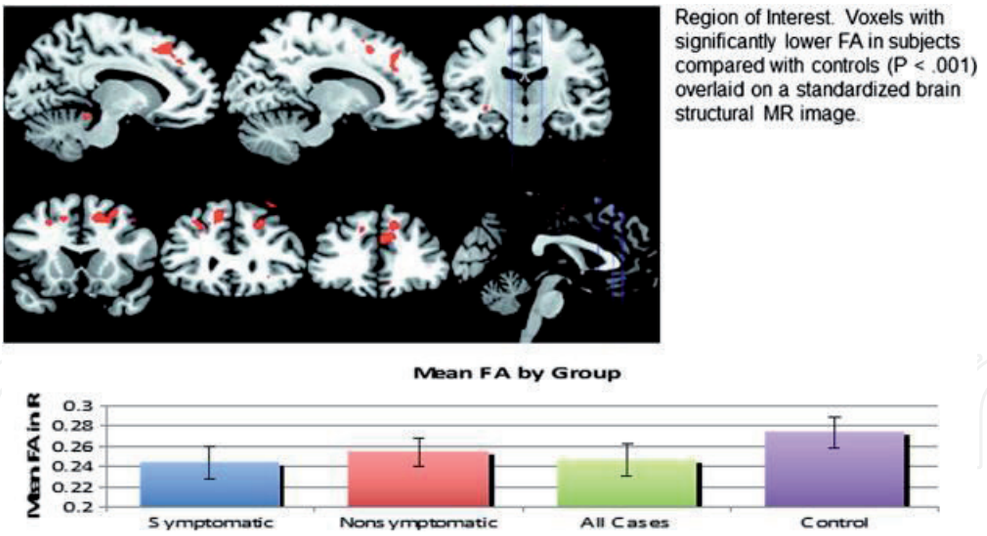

Figure 2.
Decreased FA in the anterior cingulate. The decrease in FA is most significant in patients with OTCD who are symptomatic, but note also FA decreased in those "asymptomatic" patients as compared to controls without any urea cycle disorder.

DTI was much more sensitive to changes in WM microstructural differences than fast spin echo (FSE) T2-weighted imaging for detecting abnormalities in normal-appearing WM. We also found that the degree of the abnormality correlated with degree of cognitive deficits. The location of the deficits in the frontal WM is highly significant as this area is important in the connectivity of fibers vital to executive function, working memory, and attention.

4.3 Progressive WM injury predicts cognitive decline with the most pronounced effects on processing speed and executive function

With our research we have shown DTI evidence of WM injury in motor tracts that subserve executive attention and working memory and can correlate measures of FA with specific working memory tasks. These changes result from WM tract disruption and related cortical disconnection. Quantitative data on the WM microstructure provide a more direct measurement of brain tissue integrity than standard MRI sequences.

4.3.1 WM damage in OTCD

Neuropathological findings have been extensively examined in patients who have died due to urea cycle disorders. These findings shared pathology with other more common conditions such as hepatic encephalopathy as well as hypoxic ischemic encephalopathy. Previous autopsy and, more recently, neuroimaging studies suggest that OTCD results in a predilection for WM injury. And in patients several months prior to death after surviving a neonatal presentation, neuropathological findings consisting of cortical atrophy, ventriculomegaly, gliosis with Alzheimer type II astrocytes, spongiform changes at the gray/white junction, ulegyria, and spongiform changes in the deep gray nuclei-basal ganglia and thalamus have been reported in the literature [52–55].

Neuroimaging studies which have been performed several months after a neonatal hyperammonemic event, months later in neonatal coma survivors, are consistent with these pathological findings, correlating with hypomyelination of WM, myelination delay, cystic changes of the WM, and gliosis of the deep gray matter nuclei. The original reports were small case series using clinical CT initially and then, only

later, MRI. Survivors of prolonged hyperammonemic coma had severe anatomic abnormalities including ventriculomegaly and cortical atrophy. Today, these severe findings are rarely encountered if patients are diagnosed promptly, and duration of hyperammonemia is shortened.

4.4 Functional MRI and UCD research (fMRI)

The basic premise behind fMRI is the increase in blood flow to the local vasculature that accompanies neural activity in the brain. This leads to a local reduction in deoxyhemoglobin. An increase blood flow occurs without an increase of similar magnitude in oxygen extraction.

Deoxyhemoglobin is paramagnetic; it alters the T2*-weighted magnetic resonance image signal and serves as the source of the signal for fMRI [56]. Coupling between neural activity and changes in blood flow was first reported in 1890 (Roy and Sherrington) [56]. By using fMRI, one can observe how the brain is functioning and what areas of brain are activated while a person is performing a specific task. It can allow unmasking of regional vulnerability, circuitry, and recovery of function after damage or intervention.

4.5 Magnetic resonance spectroscopy

MRS is another clinical sequence that provides noninvasive analytic method of identifying and measuring the individual brain chemicals present in various brain regions. ^{1}H–MR spectroscopy is widely used in clinical practice to provide information on brain metabolites. The major metabolites that can be seen include choline (Cho), creatine (Cr), N-acetyl aspartate (NAA), glutamine (Gln), and the osmolytes: myoinositol (mI) and taurine (Taur). Metabolites that can be detected have a unique frequency resonance that is termed the chemical shift. The chemical shift is reported as parts per million (ppm). The advantage of this measure in ppm is that it is the same at any magnetic field strength. The basis of the signal derives from Larmor frequency and coupling. The frequency of individual nuclei is compared to a reference compound called tetramethylsilane (TMS). The MRS is read from right to left. The metabolites that are disrupted in OTCD include Gln, Cho, and mI [57, 58]. One can quantitate the metabolites by either using in house software or using a commercially available program such as linear combination modeling or LCModel [59].

The magnetic resonance (MR) signal detectable is directly proportional to the concentration of the nuclei in the prescribed voxel. Because the brain is mainly composed of water which has a concentration of 55.5 mmol per gram, this must be subtracted in the analysis as the concentration of other chemicals such as NAA or PCr is on the order of 0.015 and 0.5 mmol per gram of tissue. Common voxel sizes used in MRS are from 1 to 5 mm^3.

Both the size and shape of a peak seen on a spectrum are due to the contribution of five attributes:

1. The concentration of nuclei.

2. The T1 and T2 relaxation times of the metabolite. These are also affected by the TR (relaxation time) and TE (echo time) of the MRS sequence as certain metabolites at low concentration may only be seen best at low TE.

3. Magnetic inhomogeneity across the sample. This can be corrected to some extent by a process called shimming. This implies the process by which the main magnetic field (Bo) is made more homogenous by applying small electrical

currents. This can be done passively, as many vendors have automated shimming packages on the scanners, or manually.

4. Another consideration, especially in the case of ^{1}H MRS in the UCD, is the presence of overlapping peaks since several metabolites in whole or in part may have overlapping peaks at a certain ppm.

5. Whether the line is expected to be single or a multiplet. This is determined by the chemical structure J coupling effects.

Some of these principles are explained below.

MR proton spectroscopy has great utility in evaluation of brain metabolic disturbances. Although a nonspecific pattern (elevated Cho, depressed NAA) is common in many types of brain disease, short echo time (TE) MRS (i.e., TE < 30 msec) can reveal more specific metabolic signatures. It is also useful to focus on the temporal changes of chemicals rather than only what is abnormal. Furthermore, temporal changes on subsequent exams can help support or refute the benefit of ongoing therapeutic measures. A simple single voxel technique boasts better signal-to-noise ratios (SNR) and allows shorter TE options than multivoxel technique.

Voxel size is always a consideration, since there is a balance between signal-to-noise ratio and tissue specificity; ideally, it should be as large as possible to achieve satisfactory SNR but small enough to target the area of interest. Generally, a 2 × 2 × 2 cm (2 cm^3) voxel is sufficient; voxels smaller than 1 cm^3 are unlikely to be worthy of the acquisition time it would require to achieve reasonable SNR. Voxel location and echo times should be selected based on the suspected and/or discovered disease patterns. We typically perform ultrashort (TE 14, TR 1500; STEAM technique), short (TE 35, TR 1500-2000; PRESS technique), and intermediate (TE 144, TR 1500-2000; PRESS) or long (TE 288, TR 1500-2000; PRESS) echo time sequences. Examples of metabolites that are best seen at short echo include glutamine and glutamate.

In the case of glycine at 3.55 ppm, it is necessary to obtain at least one MRS data point using an intermediate (i.e., 144 msec) or long (i.e., 288 msec) echo time to remove the spectral contamination of mI that is also around 3.5 ppm.

When are longer echo times preferred? Longer echo times improve diagnostic specificity in disorders such as maple syrup urine disease (MSUD) by eliminating the normal background macromolecular signal that can hide branched-chain amino and ketoacid peaks.

The noninvasive detection of elevated brain glutamine by ^{1}H MRS has also been shown to be a useful biomarker in chronic hepatic encephalopathy [60–62]. We have observed clinically, and it has been shown that glutamine has been implicated in hyperammonemic encephalopathy. A rise in plasma glutamine levels precedes HA [60–62]. The importance of glutamine in this process is further strengthened by the relationship between HA, neurologic dysfunction, and cerebral spinal fluid glutamine concentrations observed in patients with hepatic encephalopathy.

The UCDC presented the largest series of adult patients with OTCD who were imaged using ^{1}H MRS at 3 T and discuss the utility of advanced imaging in understanding the underlying mechanisms of dysfunction [57]. ^{1}H MRS studies have demonstrated elevations in Gln and decreases in mI and Cho in patients who are clinically symptomatic [51]. We showed with ^{1}H MRS decreased mI is also an important biomarker and also seen in females who describe themselves as asymptomatic [57]. We have hypothesized that the decrement of mI might constitute a useful biochemical marker with which to discriminate females with a partial deficiency.

4.5.1 ^{13}C MRS

Although ^{1}H MR spectroscopy is a sensitive tool to detect biochemical abnormalities in individual patients, in vivo ^{13}C MR spectroscopy can reliably be used to quantitate distinct signals from glutamate and glutamine. Unambiguous assignment of these metabolites can contribute to a better understanding of the pathogenesis and treatment of brain dysfunction in UCDs. With the use of carbon 13 (^{13}C) MR spectroscopy, abnormalities in cerebral glutamate metabolism have been noted in patients with chronic hepatic encephalopathy. Therefore, the next step was to use this technique to investigate cerebral glutamate turnover rate in patients with partial OTCD.

This method allows study of glutamate neurotransmission which is carried out by a glial neuronal process that includes the oxidation of glucose and the Gln/Glu cycle [63]. The metabolic model predicts that under conditions of elevated plasma ammonia, the increase in the rate of Gln synthesis is stoichiometrically coupled to increase in the uptake of the anaplerotic substrates CO_2 and ammonia with concurrent efflux of Gln from the brain. Furthermore, studies in hyperammonemic rats suggest that only a fraction of Gln is used to synthesize GABA via Glu. The remainder passes through the neuronal TCA cycle. Bluml et al. have previously shown that there is disturbed neurotransmitter Glu/Gln cycling in chronic hepatic encephalopathy [63, 64]. In their studies, Glu enrichment was decreased and Gln enrichment was increased.

5. Beyond ammonia: relationship of dysregulation of glutamatergic and GABAergic neurons in patients with HA and occurrence of seizures

Despite decades of research, the mechanisms leading to neural injury in HA are still not well understood. Ammonia toxicity is not necessarily an adequate explanation for the degree of cognitive dysfunction seen in patients with argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), and arginase deficiency (ARG). ASS is also referred to as citrullinemia.

Recent studies by our group and others, however, have shown that HA exposure alters several amino acid pathways and neurotransmitter systems, cerebral energy metabolism, nitric oxide synthesis, oxidative stress, and signal transduction pathways which all increase the risk for seizures [30, 65].

Epilepsy had previously been considered an infrequent manifestation in urea cycle disorders, but our longitudinal study (LS) of infants with UCD at a single site (Children's National Health System) found subclinical electrographic seizures (ES) (detected on EEG without clinical manifestations) to be surprisingly common during acute hyperammonemic episodes [66].

This unanticipated finding was particularly identified in neonates with HA. This new finding raises the question of whether seizures play an important role in the etiology of neurocognitive deficits in UCD and whether seizures could afford a biomarker that correlates with brain damage in these disorders.

We have observed ES developing in patients in whom HA rebounded following discontinuation of ammonia scavengers. During HA, the brain is vulnerable to injury as a result of increased permeability and alterations in energy metabolism. In this small cohort, we observed that children with evidence of ES had abnormal MRI scans and/or adverse neurodevelopmental outcomes. This is consistent with what is seen in animal models. The animals develop change in behavior and ataxia and ultimately seizures [67].

HA episodes are critical periods in which to intervene in order to prevent long-term cognitive disability. In neonates and children, HA episodes occur in the context of a developing brain that already has vulnerabilities as a result of normal remodeling, synaptogenesis, and ion channel development. It is also the period in life recognized to be at highest risk for seizures. It is possible that seizures in HA infants and children with UCD are an early biomarker of perturbed metabolism and, left untreated, may contribute to brain injury and subsequent intellectual and other developmental disabilities.

Considerable evidence shows that HA compromises brain energy metabolism which predisposes the patient to seizures due to neuronal depolarization in association with lower energy. The seizures then in turn further lower brain energy, setting in motion a physiologic cycle: HA → lower ATP → depolarization/increased vulnerability to seizures → frank seizures (or, at least, ES) → further lowering of ATP → more seizures (or ES) [68].

5.1 Seizures in UCD

While there is a paucity of investigation of the incidence/prevalence of seizures in UCD, one early study evaluated 11 EEG tracings of 4 infants, irrespective of clinical seizure status [69]. This small study identified epileptiform EEG alterations and hypothesized that they may be a characteristic manifestation of UCD. Later, a retrospective analysis of EEG tracings and head CT scans in 49 UCD patients revealed EEG abnormalities during a clinically stable period that were predominantly observed in patients with abnormal CT scans [70].

In agreement with these reports, our group has identified that patients with distal UCD (where ammonia levels are not as elevated as in proximal disorders) have a high frequency of epilepsy and cognitive dysfunction, raising the possibility that seizures may be associated with other biochemical abnormalities in distal UCD [36, 71]. Additionally, we have shown that disrupted neural networks underlying working memory are a consistent finding in UCD patients with mild as well as severe symptoms [25, 46]. These studies strengthen the importance of understanding the incidence/prevalence of epilepsy in patients with UCDs and if and how this may affect later cognitive function. It further suggests that patients may be undertreated if we focus solely on ammonia-lowering agents and fail to recognize and treat concurrent seizures, as there are clearly other factors, such as disturbed mitochondrial function and oxidative stress, implicated in ammonia-induced neurotoxicity [68, 72–74].

5.1.1 Animal models of UCD and seizures

Both myoclonic and tonic–clonic seizures have been reported in the OTCD spf-ash mouse, an animal model of late-onset UCD with variable phenotype and severity. One study showed that the seizures were linked to the neurotoxic effect of HA on astrocytes, which increased and desynchronized astrocytic Ca^{2+} signaling and compromised the ability to buffer extracellular potassium. Using an animal model of inducible HA, the NAGS knockout (NAGSko) mouse, develops HA within a few hours of withdrawal of effective treatment with N-carbamylglutamate and L-citrulline (NCG + Cit) [75]. Studies in these mice demonstrated that they manifested seizures during HA but unexpectedly also had seizures during baseline recording when blood ammonia levels were normal. In this study, EEG seemed to be a sensitive measure of detecting neuronal dysfunction from HA and suggested its use as an early biomarker of its damaging effects.

The common hypothesis as to how ammonia may lead to seizures is based on the idea that reducing ammonia in the blood will reduce the influx into astrocytes, thereby inhibiting the glutamine synthetase enzyme. This is the basis of ammonia lowering agents in clinical use. However, the work of Rangroo-Thrane et al. challenged that idea by showing that this only may worsen the neurological condition by increasing neuronal exposure to both [NH_4^+] and [K^+]. They have shown that failure of buffering potassium in astrocytes actually is the critical mechanism that contributes to ammonia neurotoxicity. Using awake, intact mice that were induced to have HA, this research group reported that when ammonia was blocked from entering the astrocyte via the potassium transporter Na^+-K^+-$2Cl^-$ cotransporter isoform 1 (NKCC1), seizures developed. They also conclude that a therapeutic intervention should work by blocking this pathway by inhibiting NKCC1 [75].

To determine the incidence of seizures in our UCD cohorts, we conducted a data mining study of six children who were enrolled in the UCDC since 2003 and who had continuous video EEG (cVEEG) during a HA episode and sufficient data to abstract. We accessed their data including clinical information, MRI scans, and metabolic laboratory results including ammonia and glutamine levels. We found that seizures occurred in neonates with UCD even when ammonia and glutamine levels had returned to normal. We further found that interburst interval duration (the time between brain activity and silences) correlated with ammonia levels. During periods of HA, the duration of electrical silence was prolonged, and the EEG pattern could be used to predict elevated ammonia levels (**Figure 3**). A prolonged interval was also correlated with cerebral dysfunction and an abnormal follow-up MRI showing injury (**Figure 4**) [76]. cVEEG therefore can be a useful tool for managing infants with HA and may be essential for seizure management, especially for infants in deep metabolic coma. Features of the EEG appeared predictive of short-term cognitive outcome and structural injury on MRI in this cohort.

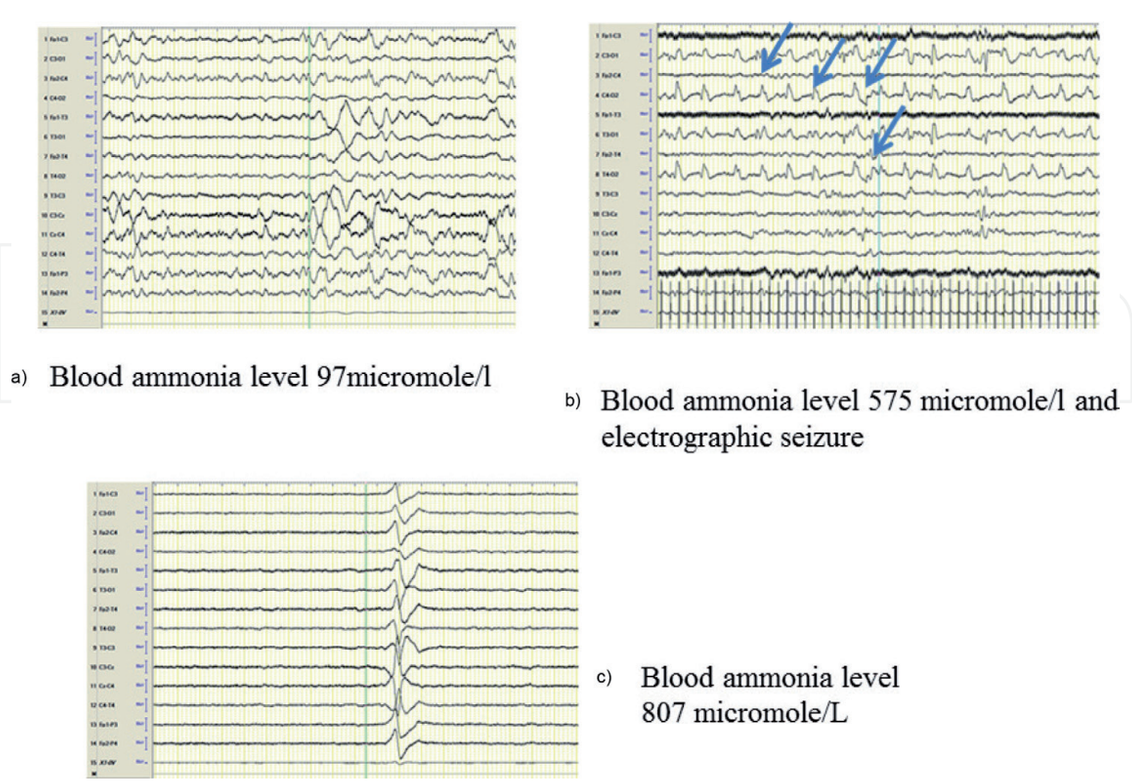

Figure 3.
EEG patterns change with concentration of ammonia from normal neonate (a) to increased interburst intervals, in this case also showing focal seizure (arrows) (b) to more significant suppression of brain activity as ammonia concentration increases further (c).

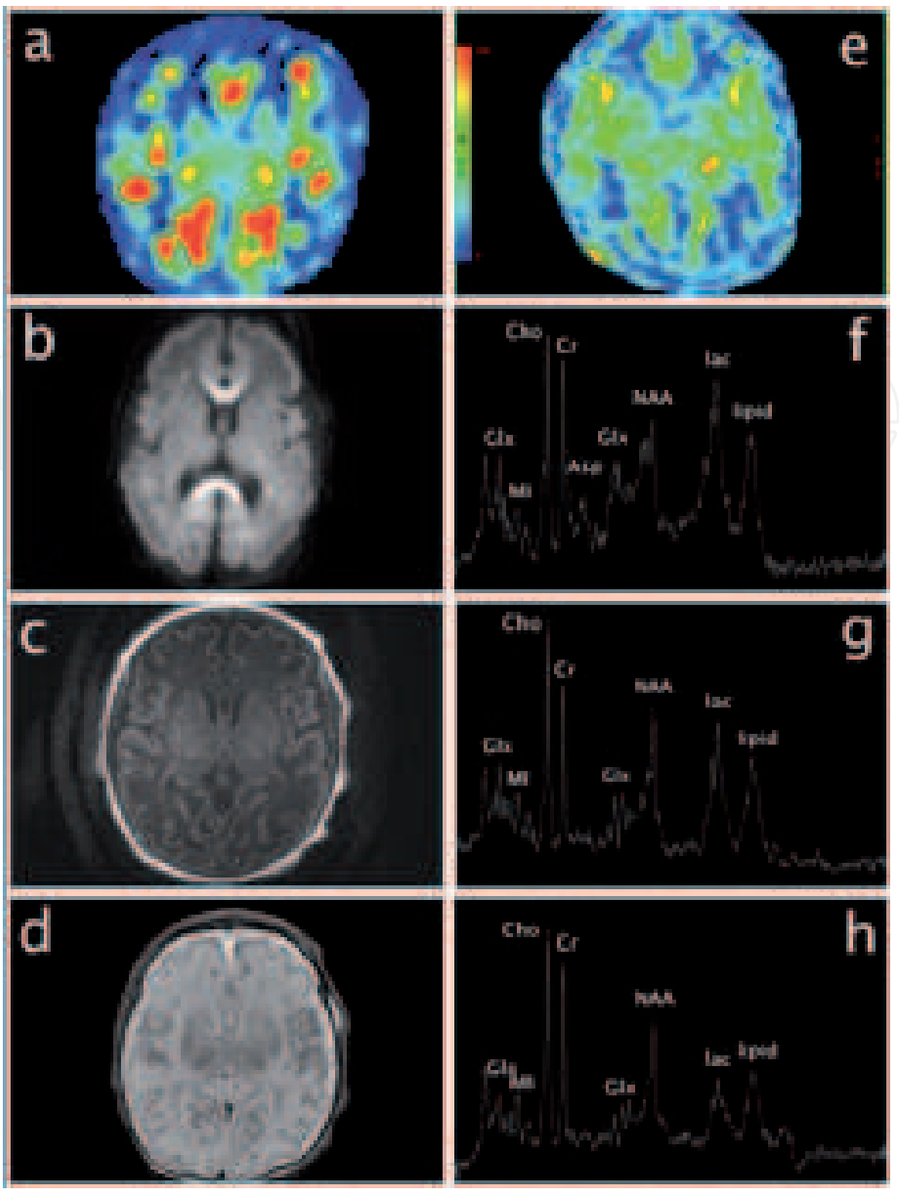

Figure 4.
Selected axial brain MR images at the level of the basal ganglia at day of life 14 (a–d) and 18 (e). Heterogeneous cerebral hyperperfusion improves over time between exams. (a and e) reduced diffusion is present showing hyperintense signal in the callosal splenium and genu, sagittal stratum, internal capsules, frontal WM, and, to a lesser extent (with partial pseudonormalization), the cerebral cortex and deep gray nuclei in correlation with the ADC map (not shown). (b) Hyperintensity on T1WI and (c) hypointensity onT2WI (d) are present extensively throughout most of the cerebral cortex, and mild signal changes are present affecting the cerebral deep gray nuclei. The cortical signal changes on the T1 and T1WI represent laminar necrosis. The unmyelinated cerebral WM demonstrates excessive T1 and T2 prolongation. There is mild diffuse cerebral volume loss with prominent sulci and ventricles.

6. Conclusions

Our understanding of the neurocognitive challenges of OTCD has been improved with the study of advanced MR imaging techniques; however, many issues remain unresolved. We are beginning to understand the neural networks impacted and have been able to correlate imaging findings with specific cognitive outcomes. We now need to scale it back to determine the earliest markers of brain injury. New findings of electrographic seizures in neonates with UCD raise questions of whether seizures play an important role in the etiology of neurocognitive deficits in UCD and whether changes on the EEG could afford a biomarker that correlates with brain changes in these disorders. Future studies are directed towards studying a more diverse group of UCD patients during baseline as well as during

HA events. Pattern recognition to inform the structural and biochemical changes on MRI will allow us to move towards precision management where neuromonitoring may inform moment to moment changes in clinical management.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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