Conference goals

- Talk about neurological problems in patients with urea cycle disorders (UCD)
  - OTCD/CPS1
  - Arginase deficiency
  - ASA, ASS
- Discuss old and new tools to study these problems
- What have we learned? The past 7 years of neuroimaging patients with UCDs
- Can we use these technologies to track or prevent injury?
Urea cycle

- Why do we/our children have neurological problems as part of UCD?
  - Is there a way to prevent it?
- The full urea cycle is in the liver, not the brain
  - Converts protein in our food to nitrogen
  - Nitrogen can make ammonia, a harmful substance or to urea which is less harmful to the body and brain
  - Makes other compounds we need in metabolism

What happens when something goes wrong with protein breakdown: get more ammonia

- High ammonia: CPS1, NAGS, OTCD, neonatal period in arginase deficiency
- When there is an early block in the urea cycle, ammonia build ups and enters the brain
  - Period of reversible brain injury
    - If recognized and treated
    - Neuroprotection
  - Irreversible brain injury
    - Once a critical time has passed
      - Loss of brain cells-neurons
      - Brain swelling

Distal disorders

- Although any defect in the urea cycle can cause hyperammonemia complete deficiencies of proximal urea cycle enzymes produce the highest levels of ammonia
- However, there are cognitive problems in patients with argininosuccinic acid synthetase (ASS) and argininosuccinate lyase deficiency (ASL) who might also have seizures
  - Factors other than hyperammonemia may contribute to poor neurocognitive outcome in the distal UCDs
Distal disorders

- Seizures, delay in development and abnormal behavior are not explained by hyperammonemia

What are the toxins in distal disorders?

- Arginine is made into a compound called guanidinoacetate
- Guanidinoacetate is transient and is immediately made into creatine which is used for energy
- Normally, guanidinoacetate is not found in brain, plasma, or urine
  - Brain guanidinoacetate was increased in a previously reported ASL patient
  - The possibility of a cerebral creatine deficiency has not been studied in patients and may represent a treatment opportunity
Distal disorders, imaging

- 1H MRS studies showed increased cerebral guanidinoacetate concentrations in ASS patients.
- The cerebral creatine content in ASS deficient patients was below normal.
- In ASL deficient patients the creatine content was above normal.
- Plasma and urine guanidinoacetate and creatine were normal.
- No clear relation was found between the results of plasma and urine guanidinoacetate and creatine in blood and urine compared to the brain.

Long term consequences

- Cognitive problems
  - If the ammonia stays elevated
    - Duration
    - Level
    - Other factors
  - Memory, organization
  - Attention/processing
    - but not true ADHD
  - Fine motor skills

Executive dysfunction in OTCD

- Our research and that of others has shown that "asymptomatic" OTCD is associated with:
  - Altered chemical profile deficits in cognitive functions in the front of the brain (prefrontal cortex) (PFC)
    - Working memory
    - Executive cognition
    - Reaction speed
  - These deficits contribute significantly to disability in OTCD despite normal global IQ.
Working memory

- The storage, manipulation and retrieval of information in conscious awareness over brief intervals
- Critical component of executive cognition and is known to be impaired in OTCD

Neuroanatomical substrates of memory

- A network of brain structures control working memory
- Dorsolateral prefrontal cortex (DLPFC)
- Regulates the encoding and manipulation of memory items

- DLPFC activation follows an inverted U-shaped curve in response to increases in cognitive load (difficulty of task)
  - Activation peaks when task demands maximally tap an individual’s capacity
  - Decreasing activation after capacity is breached
Neuroanatomical substrates of memory

- Inefficient DLPFC activation
  - when an individual reaches peak activation at low levels of cognitive demand, relative to the normal population
- Schizophrenia
- Healthy aging

N-back working memory task

- Subjects encode, maintain and retrieve letters presented in a sequence on a screen inside the MRI scanner, indicating when a letter matches the letter 2 n back in the sequence.
- Task difficulty can be modulated from 1-back (easiest) to 3-back (hardest)

2-back example:
On screen: H R J S J B D W D
Response: n n y n n n y

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What is ammonia and what does it do to brains

- Change in behavior
- Lethargy
- Seizures
- Coma
- Affects synapses or connections between cells

What happens when something goes wrong with protein breakdown and handling of ammonia??

- Toxicity: other compounds
  - Arginase deficiency
  - Too much arginine
    - Cerebral palsy type of clinical symptoms, mildest injury of UCD
    - May be toxicity from other compounds: guanidino acetate
    - Elevated arginine could augment nitric oxide (NO) synthesis and lead to oxidative damage

- ASA
  - Argininosuccinic acid
  - Seizures
  - Deficiencies: citrulline, protein: branch chain amino acids
Major unanswered question(s)

- How does having a urea cycle disorder cause problems in the brain?
  - When does the damage occur?
  - How much of the damage is reversible?
    - When?
    - What can we do to know this and prevent this
  - Is there a way to predict and prevent brain injury from urea cycle disorders?
    - First episode
    - Subsequent

Major unanswered questions

- We studied the effects of urea cycle disorders on the brain using MRI
  - Repeatable over time
  - Closely related to the body part of interest
  - Resulted in interesting findings that may change treatments
  - OTCD and arginase deficiency

Symptoms

Patients with urea cycle disorders may have cognitive

- Proximal disorders: CPS1, OTCD, NAGS
- The severity of the condition depends upon the following:
  - Enzyme deficiency: how much is working
  - How long the ammonia is elevated
  - Time between symptoms and treatment
  - Other associated medical issues
  - Age of onset and stage of brain development
Age of onset and UCDs: Neonatal

- Potential for severe neurological outcome in infants who survive hyperammonemic coma
- Neonatal onset disorders show
  - Brain atrophy (loss of brain, neurons, cells)
  - Enlargement of ventricles (fluid components)
  - Abnormal white matter (connecting fibers)
  - Cystic changes of WM (cavities filled with fluid)

Age of onset and UCDs: childhood

- Very mild to severe developmental delay, intellectual delays/mental retardation, attention, and behavioral problems
- Later onset disorders
  - Reversible white matter lesions are observed
  - Working memory, attention, fine motor, reaction time

Age of onset and UCDs: adults

- May be misdiagnosed or undiagnosed
- Thought to have psychiatric disorders
- Misdiagnosis as alcoholics (high ammonia)
- May present with coma after illness or after surgery, steroids
Clinical symptoms of UCDs

- Typical OTC
  - Lethargy
  - Unsteadiness
  - Seizures
  - Appetite is poor
  - Vomiting
  - Hypothermia
  - Coma
  - Asterixis/flapping tremor

- Partial OTC deficiencies
  - Protein aversive
  - Hyperactive behavior
  - Self injurious behavior
  - Vomiting, consciousness
  - Strokes
  - Psychiatric symptoms

Steps the brain takes when ammonia is high

- Ammonia enters the brain
  - The glial cell takes up glutamate
  - Glial cells makes glutamine

- Glutamine allows water into the cells
  - The brain swells

- Brain swelling may cause changes in level or alertness, seizures or coma
Ammonia and the brain

- When the brain is swollen for a long time
  - Brain cells die
  - Cognitive damage expected because cells are lost
- In UCDs, the damage doesn’t affect all parts of the brain equally
  - Affects areas involved in working memory and attention
  - Routine MRI may be normal even though damage has occurred
  - Need other ways to measure this damage by looking at brain biochemistry and function

Brain biochemistry markers: Glutamine

- May be a marker of injury
  - Levels increase before ammonia
    - Remains high until ammonia begins to normalize
  - Correlation poor between blood ammonia and glutamine levels
  - Plasma glutamine levels do not predict brain metabolism
    - Glutamine in brain may be higher

Brain biochemistry markers: Myoinositol

- When brain glutamine increases, it makes the water in the brain increase and changes volume
  - There has to be mechanism to keep brain volume fairly constant
- Myoinositol is a small sugar that is counter regulatory and its concentration decreases when glutamine increases
  - We can detect glutamine and myoinositol levels in brain using special imaging methods
Routine MRI tells us little about brain injury in urea cycle disorders because it usually looks normal.

T2 Axial images
FLAIR images
Show changes in the white matter of UCD patients

Diffusion Tensor Imaging (DTI)

- MR imaging technique in which blackness and whiteness of the MRI image (contrast) is based on differences in the movement (diffusion) of water molecules
  - Motion of a water molecule is random
  - Water cannot move/diffuse freely if there are boundaries
- Pattern of diffusion of water in cells
  - Can tell if the white matter is damaged
Types of water movement (diffusion)

- Isotropic diffusion
- Restricted diffusion

Open pool of fluid (Ventricles)
No restriction (ventricles)
Diffusion in an Axon
Restricted environment (an axon)
Myelinated axons

Diffusion Images

- Raw diffusion images
- Tractography
- Directionality
- Isolation of fiber tracks
Diffusion tensor imaging shows evidence of white matter injury in motor tracts that connect parts of the brain important in attention and memory.

New data

- Suggests significantly decreased white matter in OTCD versus individuals without OTCD
- When mapped to white matter atlas the pathways involved in
  - Coordination
  - Attention and memory

There were differences in scores on tests of memory and attention that could distinguish subjects and controls.

- Stroop Performance Group Means
Effects of UCD on the Brain

DTI in arginase deficiency
- DTI changes specific for OTCD
- DTI shows differences in arginase deficiency
  Corticospinal tracts
  Spastic, stiff legs
fMRI and brain mapping

- Short for "functional magnetic resonance imaging"
- fMRI allows researchers to look at events instead of just structures
- Color changes on fMRI scans show researchers which part of the brain is active when a subject performs a mental task such as speaking, listening to a bell ring, or solving a math problem.
fMRI

- Researchers may get a good idea of what learning "looks" like
- Measure it in children far earlier than standardized tests allow

**Functional magnetic resonance imaging (fMRI)**

- Technique for measuring brain activity
- It works by detecting the changes in blood oxygen and flow that occur in response to neural activity
- When a brain area is more active it uses more oxygen
  - Blood flow increases to the active area
  - fMRI can be used to make activation maps showing which parts of the brain are involved in a particular mental process
**Functional MRI**

- Magnetic resonance imaging can be used to map changes in brain blood flow that correspond to doing an activity (visual, motor, thinking)
- Ability to directly observe brain function
  - Advance our understanding of brain connection, organization and how diseases may alter this
  - How one recovers from brain injury

**fMRI**

- By using fMRI, one can observe how the brain is functioning and what areas of brain are involved while a person is performing a specific task
N-Back fMRI task: 1-back vs 2-back

1-back   n e g h g k k

2-back   c w c h f p f

Results

Set up
Magnetic resonance spectroscopy— a snapshot of brain biochemistry

- **NAA**: Marker of neuronal number and integrity, may be decreased in disorders that damage neurons.
- **Lactate**: May be elevated in disorders that impair energy metabolism.
- **Creatine**: Marker of energy status.
- **Choline**: Marker of integrity of nerve cell membranes, elevated with membrane damage.
- **Myo-inositol**: Involved in cell signaling, may indicate cell volume.
- **Glutamate**: Excitatory amino acid neurotransmitter, precursor for GABA, inhibitory neurotransmitter.
- **Glutamine**: Storage depot for ammonia.

MRS—A type of brain scan that can detect abnormalities in the brain’s biochemical processes.
Future directions in metabolic imaging

- Combine different imaging datasets and hardware to exploit the synergy of various imaging technologies
  - Functional imaging
  - Functional DTI
  - Spectroscopic DTI
  - Multinuclear imaging
  - Spectroscopic imaging: regional metabolite maps at short TEs
  - PEPSI
  - New modalities

Future projects

- Acute changes and recovery from HA
  - What happens in the first week of a HA episode and how does the brain recover?
  - Multiple UCD sites
  - Anatomy, MRS
  - Possibly DTI, fMRI
  - Eligible: Ages 7-60 years after HA due to any reason

fMRI after hyperammonemia

- Type and location of brain activity
  - at baseline
  - after HA
  - At various time points after HA recovery
  - Versus controls
fMRI after HA

- Aberrant activation from expected pattern
  - Evidence of localized injury to part of brain underlying a motor or cognitive function
    - Is it permanent?
    - Possibility for recovery?
    - Implications for educational programs
    - Implications for treatment

Who is eligible to participate??

- OTC females and late onset males, confirmed biochemically or genetic mutation
- Ages 7-60 years of age
- English speaking
  - Able to comply with testing
  - Not claustrophobic
- Stable health
  - No significant illness at time of study
  - Not pregnant
  - Agree to take a pregnancy test if female and of child bearing potential
- No metal in body
  - New tattoos, pacemakers, pins, clips, pumps, cochlear implants, permanent eyeliner, metal fragments in the eyes, etc.
  - Not on too many medications which might interfere with the studies

What do I have to do??

- Travel to Washington, D.C. for 2 days
- Undergo 1 ½-2 hours of cognitive testing
- Undergo 4 hours of scanning
  - Split over 2-3 sessions
Pasadena studies with Dr. Ross

- 6 subjects with partial OTCD and compared to control, unaffected subjects
- 13C MRS
- Intravenous low dose infusion of 0.23g/kg body weight of 99% $^{13}$C glucose (20% W/V) over 10 minutes

Labeled glucose appeared in the brain of all subjects.
Brain glutamate synthesis from glucose is significantly reduced in OTCD
On going studies needed
New studies: Near infrared spectroscopy

- Optical technique for measuring blood oxygenation in the brain
- It works by shining light in the near infrared part of the spectrum (700-900nm) through the skull
  - Detect how much the remerging light is reduced
  - How much the light is attenuated depends on blood oxygenation
  - NIRS can provide an indirect measure of brain activity

Advantages of NIRS

- Younger age
- More cognitively impaired
- Claustrophobia

Newer methods

Subject sits at computer screen with optical emitters and detectors affixed over region of the brain under study. The apparatus is completely non-invasive, and allows brain activity to be monitored while subjects are engaged in a cognitive task.
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