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Investigating neurological deficits in carriers and affected patients with ornithine transcarbamylase deficiency

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ABSTRACT

Background: : Urea cycle disorders are caused by dysfunction in any of the six enzymes and two transport proteins involved in urea biosynthesis. Our study focuses on ornithine transcarbamylase deficiency (OTCD), an X-linked disorder that results in a dysfunctional mitochondrial enzyme, which prevents the synthesis of citrulline from carbamoyl phosphate and ornithine. This enzyme deficiency can lead to hyperammonemic episodes and severe cerebral edema. The objective of this study was to use a cognitive battery to expose the cognitive deficits in asymptomatic carriers of OTCD.

Materials and methods: : In total, 81 participants were recruited as part of a larger urea cycle disorder imaging consortium study. There were 25 symptomatic participants (18 female, 7 male, 25.6 years ± 12.72 years), 20 asymptomatic participants (20 female, 0 male, 37.6 years ± 15.19 years), and 36 healthy control participants (21 female, 15 male, 29.8 years ± 13.39 years). All participants gave informed consent to participate and were then given neurocognitive batteries with standard scores and T scores recorded.

Results: : When stratified by symptomatic participant, asymptomatic carrier, and control, the results showed significant differences in measures of executive function (e.g. CTMT and Stroop) and motor ability (Purdue Assembly) between all groups tested. Simple attention, academic measures, language and non-verbal motor abilities showed no significant differences between asymptomatic carriers and control participants, however, there were significant differences between symptomatic and control participant performance in these measures.

Conclusions: : In our study, asymptomatic carriers of OTCD showed no significant differences in cognitive function compared to control participants until they were cognitively challenged with fine motor tasks, measures of executive function, and measures of cognitive flexibility. This suggests that cognitive dysfunction is best measurable in asymptomatic carriers after they are cognitively challenged.

1. Introduction

Urea cycle disorders (UCDs) result from deficiencies in any of six enzymes and two transport proteins involved in the urea cycle or synthesis of urea. Ornithine transcarbamylase deficiency (OTCD) results from a mutation in the ornithine transcarbamylase mitochondrial enzyme that normally catalyzes the synthesis of citrulline from carbamoyl phosphate and ornithine [1]. It is the only urea cycle disorder that is X-linked, and as a result, males and females are differentially affected [2–5]. The true incidence of this disorder is unknown, due to its rarity, however the estimated combined incidence for all UCDs ranges from 1 in 8200 to 1 in 30,000 [1,6].

A deficiency of ornithine transcarbamylase leads to an excess of ammonia being generated by the urea cycle instead of urea [1]. Elevation of ammonia alters several amino acid pathways and neurotransmitter systems, interferes with cerebral energy metabolism, nitric oxide synthesis, oxidative stress and signal transduction pathways. The only route of ammonia disposal is via the glutamine synthesis pathway, generating an excess of glutamine in the brain, and astrocytes are the only cellular compartment in the brain capable of glutamine (gln) synthesis. These high levels of glutamine are believed to cause a shift in osmotic gradient within the brain, causing excessive fluid to cross the blood brain barrier, leading, often, to severe edema [1].

Abbreviations: UCD, urea cycle disorder; OTCD, ornithine transcarbamylase deficiency; CTMT, Comprehensive Trail Making Test; WASI, Wechsler Abbreviated Scale of Intelligence; ANCOVA, analysis of covariance; WIS, Wechsler Scale of Intelligence; BRIEF, Behavior Rating Inventory for Executive Function; WJ-III, Woodcock–Johnson Intelligence Scale; VMI, Beery–Buktenica Developmental Test of Visual-Motor Integration; PPVT-III, Peabody Picture Vocabulary Test — Third Edition; EVT, Expressive Vocabulary Test.

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Although not universally accepted, gln is a prime suspect in the list of neurotoxins associated with the neurological aspects of OTCD. Vomiting, lethargy, and coma can characterize severe episodes of hyperammonemia; however, mild cases often go unrecognized and undetected. If uncontrolled or untreated this can lead to episodic encephalopathy and ultimately result in brain injury and death [1,7].

Many have investigated the cognitive insults resulting from hyperammonemic encephalopathy in OTCD [2–4]. Our study examined the effects of OTCD on motor skills, simple and complex attention, executive function, verbal and nonverbal memory, and language skills in a cohort of children and adults with OTCD ascertained due to having an affected sibling, father, or other family member. Those who participated were enrolled in an NIH funded neuroimaging study as part of the Urea Cycle Rare Disorders Consortium. Our study offers a unique perspective on cognitive deficits in OTCD because there is a wide range of ages (7–60 yrs) and participant scores were stratified by asymptomatic carriers, symptomatic participants, and an age and gender-matched control population, which is often not possible due to the rarity of this disorder. The goal of this study was to elucidate potential cognitive tasks that were more sensitive to the cognitive deficits in carriers of OTCD.

2. Materials and methods

2.1. Participants

Participants with OTCD, both symptomatic and asymptomatic carriers, were recruited through the Online Rare Diseases Clinical Research Network registry, the National Urea Cycle Disorders Foundation, the Society for Inherited Metabolic Disease, and colleagues of the principal investigator known to service OTCD patients in metabolic clinics across the country. Participants with OTCD had molecular confirmation if available or clinical phenotype with symptoms including protein intolerance, emesis, or unexplained encephalopathy or psychiatric disease with appropriate biochemical findings. Participants were stable at the time of the evaluation and had normal ammonia levels. Asymptomatic carriers were relatives of affected individuals recruited for this study. One-third reported dietary protein aversion.

Control participants were matched to OTCD participants based on age and gender. Control participants were recruited via IRB-approved advertisements posted throughout the Georgetown University Hospital, medical school, and graduate school. All were consuming a normal diet. Individuals with a past medical history of epilepsy, stroke, cognitive dysfunction, liver disease, psychiatric illness, or who scored below 80 on the WASI were excluded from this study. Participants were at baseline health at the time of the study (i.e. not directly before or after a HA).

Participants with OTCD, who had molecular confirmation were subdivided into symptomatic or asymptomatic groups based on results of previous stable isotope studies, if available, or clinical phenotype with symptoms including protein intolerance, emesis, or unexplained encephalopathy or psychiatric disease with appropriate biochemical findings. Overall, there were 81 participants with an age range of 7–60 years (Table 1). There were 25 symptomatic participants (18 female, 7 male, 25.6 years ± 12.72 years), 20 asymptomatic participants (20 female, 0 male, 37.6 years ± 15.19 years), and 36 healthy control participants (21 female, 15 male, 29.8 years ± 13.39 years), who gave informed consent. The informed consent was approved by the Children's National Medical Center Biomedical Institutional Review Board and given to all participants prior to performing the experiment.

Table 1
Age distribution of study participants, showing also percent of subjects of each age range.

Age range (yrs)	Percent of population (%); N
5–10	6 (5)
11–19	15 (12)
20–29	31 (25)
30–39	19 (15)
40–49	11 (9)
50–59	17 (14)
60+	1 (1)

2.2. Cognitive assessment

We investigated neurocognitive performance differences between OTCD participants (symptomatic participants and asymptomatic carriers) and control participants using the assessment battery detailed below. All scores were normalized to age allowing comparison across age groups (Table 2).

2.2.1. Assessment of executive function

2.2.1.1. The Stroop. The Stroop is used to assess executive function levels through measurements of processing speed, simple and complex reaction time, speed–accuracy trade off, and inhibition and selective attention. Participants are required to indicate the color of a word flashed on a screen. The words, "red," "blue," or "green," are displayed in either the corresponding color (congruent trials, e.g. "red" is written in the color red) or a conflicting color (incongruent trials, e.g. "blue" is written in the color green) [8].

2.2.1.2. The Comprehensive Trail-Making Test (CTMT). The CTMT is a measure of set-shifting, working memory, divided attention, and cognitive flexibility. Numbers are presented as Arabic numerals (e.g. 1, 8) or spelled out in English (e.g. three). CTMT trials 1–3 require only simple sequencing skills. Trails 4 and 5 (part B), in contrast, require a higher level of "set shifting" or cognitive flexibility analysis. Trail 4 introduces both numerical and lexical number stimuli as targets requiring the participant to locate targets regardless of appearance. CTMT Trail 5 requires the examinee to connect a series of numbers and letters in a specific sequence as quickly as possible without crossing lines [9].

2.2.1.3. The Behavior Rating Inventory for Executive Functioning (BRIEF). The BRIEF is a self-report measure that captures the individual's self-perceived executive function. Participants are required to complete a series of questions that assess the individual on eight clinical scales (Inhibit, Shift, Emotional Control, Initiate, Working Memory, Plan/Organize, Organization of Materials, and Monitor). Individuals are then scored on two indices: the Behavior Rating Index (BRI), which measures an individual's ability to control his or her behavior and emotional responses and the Metacognitive Index (MI), which measures an individual's ability to systematically solve problems through planning and organizing in a variety of contexts [10].

2.2.1.4. Digit span backwards as part of the Wechsler Intelligence Scale (WIS). The digit span backwards task measures a participant's working-memory capacity, attention, information manipulation abilities, and the ability to remember multiple pieces of information. For the digit span backwards, participants are presented orally with a series of digits (e.g., '1, 7, 9') and must immediately repeat the series of digits in the reverse order. Those able to repeat the series back correctly are then given a longer series of numbers until they are unable to successfully complete the task [11,12].

2.2.2. Verbal Memory

2.2.2.1. The Wechsler Abbreviated Scale of Intelligence (WASI) — Verbal Subtest. The WASI Verbal IQ (including the Vocabulary and Similarities tasks) is an assessment of verbal memory. The WASI Vocabulary task measures word knowledge, verbal concept formation, and fund of knowledge. The Vocabulary task consists of 38 items that the examiner

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