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CLINICAL RESEARCH ARTICLE

Relationship between longitudinal changes in neuropsychological outcome and disease biomarkers in urea cycle disorders

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BACKGROUND: Urea cycle disorders (UCDs) cause impaired conversion of waste nitrogen to urea leading to rise in glutamine and ammonia. Elevated ammonia and glutamine have been implicated in brain injury. This study assessed relationships between biomarkers of metabolic control and long-term changes in neuropsychological test scores in participants of the longitudinal study of UCDs. The hypothesis was that elevated ammonia and glutamine are associated with neuropsychological impairment.
METHODS: Data from 146 participants who completed 2 neuropsychological assessments were analyzed. Neuropsychological tests that showed significant changes in scores over time were identified and associations between score change and interim metabolic biomarker levels were investigated.
RESULTS: Participants showed a significant decrease in performance on visual motor integration (VMI) and verbal learning immediate-recall. A decrease in scores was associated with experiencing interim hyperammonemic events (HAE) and frequency of HAE. Outside of HAE there was a significant association between median ammonia levels ≥50μmol/L and impaired VMI.
CONCLUSION: VMI and memory encoding are specifically affected in UCDs longitudinally, indicating that patients experience difficulties when required to integrate motor and visual functions and learn new information. Only ammonia biomarkers showed a significant association with impairment. Preventing HAE and controlling ammonia levels is key in UCD management.

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IMPACT:

- The Beery-Buktenica Developmental Test of Visual-Motor Integration (Beery VMI) and List A Trial 5 of the California Verbal Learning Test (CVLT) may be good longitudinal biomarkers of treatment outcome in urea cycle disorders (UCD).
- This is the first report of longitudinal biomarkers for treatment outcome in UCD.
- These two biomarkers of outcome may be useful for clinical trials assessing new treatments for UCD. These results will also inform educators how to design interventions directed at improving learning in individuals with UCDs.

INTRODUCTION

The urea cycle converts waste nitrogen to urea, thus preventing toxic accumulation of ammonia. Neonates with an inherited complete deficiency of one of the urea cycle enzymes usually present with severe hyperammonemic coma within the first week of life. Individuals with a partial deficiency can present with clinical symptoms at any age depending on the degree of the deficiency and the severity of catabolic stress. After diagnosing a urea cycle disorder (UCD), long-term treatment consists of a protein-restricted diet combined with nitrogen scavenger therapy.

In the 1980s, neonatal hyperammonemic coma due to UCD had a mortality rate of close to 50%, and most of the survivors had one or more developmental disabilities and markedly reduced IQ scores.[1] The introduction of nitrogen scavenger therapy for the long-term treatment of UCDs markedly improved the one-year survival rate of patients with a complete block in the urea cycle to close to 90%.[1] How much long-term nitrogen scavenger therapy improved morbidity was not known.

In 2003, the NIH funded the Rare Disease Clinical Research Network (RDCRN) to advance the understanding and care of rare disorders. The Urea Cycle Disorders Consortium (UCDC) was one of the first consortia of the RDCRN funded. A focus of the UCDC was to perform a longitudinal, multicenter, observational, natural history study. An important objective of this longitudinal study (LS) was the identification of biomarkers for metabolic control and the evaluation of their relationship to UCD outcomes, especially neuropsychological function.

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During UCD management, an increase in nitrogen load is usually associated with an increase in plasma glutamine level. Blood ammonia levels rise with a continued nitrogen load. Both ammonia and glutamine are thought to be causative agents of hyperammonemic encephalopathy.[2] Their plasma levels are the two most important biomarkers of nitrogen load measured in the clinical laboratory. A glutamine level above 1000 µmol/L and an ammonia level above 70 µmol/L are viewed as indicators of an impending hyperammonemic event (HAE) that require treatment adjustment.[3,4]

This analysis, using data from the UCDC longitudinal study, investigates whether there is a decline in neuropsychological function during the long-term treatment of UCD and whether there is an association between biomarkers of metabolic control (ammonia and glutamine) and neuropsychological function. The hypothesis being tested was that increased nitrogen load results in elevated glutamine and ammonia levels and that these elevated levels are biomarkers that are associated with neuropsychological decline.

An initial report of neuropsychological outcome from the LS of the UCDC involved a cross-sectional analysis of the baseline assessments of participants.[5] It showed that of children with a UCD under 3 years of age, only 8% had developmental quotients in the impaired range, but that there was a significant difference in performance depending on a history of hyperammonemic episodes. In contrast, for the preschool (3–5 years) and school age group (> 5 years) half of the patients with neonatal onset disease and a quarter of the late onset group had a full-scale IQ (FSIQ) in the intellectual disability (ID) range. Possible reasons suggested for the difference in cognitive function in the under 3-year-old versus older children groups were: 1) recently improved detection and management in the newborn and infancy period or 2) ongoing neurocognitive stagnation, slower development, or decline over time. Ah Mew et al.[6] subsequently reported that two-thirds of LS participants with neonatal onset UCD who were ≥4 years old had ID. In contrast those under 4 years of age had mean developmental quotient score in the borderline normal range, similar to the findings in the earlier study[5]. It was thus important to determine whether there is ongoing cognitive impairment during long-term treatment of UCDs to date and if so, to identify metabolic biomarkers that are associated with neuropsychological outcome measures.

PATIENTS AND METHODS

Study participants

Individuals with the following 8 UCDs were enrolled in the LS of the UCDC: N-Acetylglutamate synthase (NAGS) deficiency, carbamoyl phosphate synthetase I (CPSI) deficiency, ornithine transcarbamylase deficiency (OTCD) argininosuccinic acid synthetase deficiency (ASD), argininosuccinic acid lyase deficiency (ALD), arginase deficiency (ARGD), citrin deficiency, and hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome. NAGS deficiency, CPSI deficiency, and OTCD are also called proximal UCDs, while ASD, ALD, ARGD, citrin deficiency, and HHH syndrome are also called distal UCDs. The inclusion criteria for enrollment into the LS with a specific UCD diagnosis have been previously published.[7] Clinical data, laboratory values, neuropsychological assessments (NPAs), and health-related quality of life data were collected from participants at prespecified intervals[8] (Table 1). For the study presented here, data from participants 3 years of age or older who were enrolled beginning in February 2006 and had undergone 2 NPAs by June 2018 were analyzed. These participants were chosen for analyses because of the comparability of neuropsychological tests used in subjects 3 years of age and older. When a patient underwent liver transplantation the last NPA before liver transplantation marked the end of participation in this study because metabolic biomarkers change after liver transplantation. Study subjects also included heterozygous females with asymptomatic OTCD, defined as having a heterozygous pathogenic variant in the OTC gene but never having experienced a hyperammonemic event (HAE) and not receiving treatment for UCD. These asymptomatic subjects were identified because they were relatives of affected study participants. The LS was approved by the IRBs at all participating sites and informed consent/assent was obtained from all study participants or legal guardians.

Biochemical data

Ammonia and glutamine levels at routine study visits and during HAEs were obtained from medical records. They were measured by standard methods in a clinical laboratory. Study visits where biochemical testing was performed occurred every 6 months in children and adolescents, i.e., depending on age at enrollment there were 7–9 planned study visits between the NPA at 4 and 8 years of age, 13–15 planned study visits between the assessment at 8 years of age and the assessment at 15 years of age, and 5–7 planned study visits between the assessment at 15 years of age and the one at 18 years of age; over 18 years of age, study visits occurred annually. HAE was defined as a plasma ammonia level of 100 µmol/L or higher with clinical symptoms such as irritability, lethargy, altered mental status. The interim time-period between the initial NPA and the second NPA was the period of metabolic control evaluation; the length of the period varied depending on age of patient.

The following biomarker variables were assessed: Experiencing HAEs (yes/no), frequency of HAEs (1–3; 4 or more), i) during HAEs: median of peak ammonia levels ≥300 µmol/L versus 100–299 µmol/L, versus no HAE, cumulative peak ammonia exposure across all HAEs expressed as the sum of the peak ammonia levels during HAEs, median peak glutamine level ≥1200 µmol/L versus median peak glutamine levels <1200 µmol/L; ii) outside of HAEs, i.e., during long-term treatment, assessments included: Median ammonia levels ≥50 µmol/L versus <50 µmol/L and median ammonia levels ≥70 µmol/L versus <70 µmol/L, as well as median glutamine levels ≥800 µmol/L versus <800 µmol/L, and ≥1000 µmol/L versus <1000 µmol/L. The latter variables and their cut offs were chosen because the upper level of normal for ammonia is 35–50 µmol/L and the upper level of normal for glutamine is 700–800 µmol/L depending on laboratory. A glutamine level of 1000 µmol/L and an ammonia level of 70 µmol/L (i.e., twice normal) were chosen because they are cut offs where treatment adjustments are recommended.[3,4]

Neuropsychological assessments (NPAs)

Subjects were administered age-appropriate NPAs according to the protocol for the LS of the UCDC at designated intervals based on age[8] (Table 1). At least 3 months had to have passed since the last hyperammonemic crisis for testing to occur. NPAs covered seven functional domains including global functioning, language, visual skills, motor skills, attention/executive functioning, memory, and emotional/behavioral functioning (Table 1). Those participants with IQ of less than 70 were only administered IQ, motor function, and parent questionnaire (Adaptive Behavior Assessment System (ABAS), Behavior Rating Inventory of Executive Function (BRIEF), Child Behavior Checklist (CBCL)) assessments. For individuals where the test was not available in their native language, testing of nonverbal abilities only was completed.

Neuropsychological tests that assessed the same functions, performed from 3 to over 18 years of age were considered comparable with each other and with themselves. For instance, the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) and the Wechsler Abbreviated Scale of Intelligence (WASI) were considered comparable (Table 1). Baseline NPA occurred at any age, Table 2 shows age at enrollment and at respective baseline NPA as well as the timing and age-specific NPA used for the follow-up assessments. Only participants with 2 assessments between 3 and over 18 years of age were included in this analysis.

Informatics and statistical methods

Demographic and clinical data, including the occurrence and timing of liver transplantation, as well as data on biomarkers of metabolic control and change in scores across sequential pairs of NPAs were used to evaluate the relationship between metabolic control and change in neuropsychological outcomes in study participants.

NPAs across seven functional domains (Table 1) produced age-based, standardized scores. These scores were rescaled to Standard Scores with a mean of 100 and a standard deviation (SD) of 15 for comparability. T-scores and z-scores were rescaled separately to a mean of 100, SD 15 using respective conversions. Re-scaling was done to achieve a common meaning of change across tests and age groups for comparisons without altering the fundamental relationships being studied.

The difference in test scores between first and second NPA was used as the measure of change. There are only 2 assessments per participant in this study, the first assessment at 3 years of age or older and the subsequent assessment. Analysis proceeded in steps: In the first step those neuropsychological tests that demonstrated significant median change

Table 1. Neuropsychological tests used to assess study participants by domain.

DOMAIN	Construct	TEST		
		3–5 Years of Age	**6–16 Years of Age**	**17 Years and older**
GLOBAL FUNCTIONING	Intellectual Functioning	WPPSI-III[a] Full Scale IQ[b]	WASI[c] Full Scale IQ	WASI Full Scale IQ
	Adaptive Functioning	Adaptive Behavior Assessment System, General Adaptive Composite (ABAS GAC, Parent/ Informant Report)		
LANGUAGE	Verbal Reasoning	WPPSI-III Verbal IQ	WASI Verbal IQ	WASI Verbal IQ
VISUAL SKILLS	Nonverbal Reasoning	WPPSI-III PerformanceIQ	WASI PerformanceIQ	WASI PerformanceIQ
	Visual Motor Skills	Beery[d] Test of Visual Motor Integration (VMI)		
	Visual Perceptual Skills	Beery Test of Visual Perception (VP)		
MOTOR SKILLS	Fine Motor Speed and Dexterity	Grooved Pegboard (Lafayette) - Dominant and Nondominant Hands		
	Grip Strength	Grip Strength (Dynamometer) - Dominant and Nondominant Hands		
ATTENTION/EXECUTIVE SKILLS	ADHD[e] symptoms	Attention Problems (Child Behavior Checklist (CBCL))		
	Sustained Attention	NA	Test of Everyday Attention for Children (TEA-Ch) Score!	NA
	Impulsivity	NA	TEA-Ch Walk, Don't Walk	NA
	Working Memory	BRIEF[f] Working Memory (Parent or Informant report)		
		BRIEF-P[g] Working Memory	BRIEF-2[h] Working Memory	BRIEF-A[i] Working Memory
	Flexibility	BRIEF Shift (Parent or Informant report)		
		BRIEF-P Shift	BRIEF-2 Shift	BRIEF-A Shift
	Problem Solving Efficiency	Tower of London (TOL)- Drexel Edition		
		NA	Total Move Score	Total Move Score
MEMORY		California Verbal Learning Test (CVLT-Children's Version (CVLT-C) or CVLT-II[k])		
	Immediate Recall/ Encoding	CVLT-C List A Total 1-5 (age 5 only)	CVLT-C List A Total 1–5	CVLT-II List A Total 1–5
		CVLT-C List A Trial 1 (age 5 only)	CVLT-C List A Trial 1	CVLT-II List A Trial 1
		CVLT-C List A Trial 5 (age 5 only)	CVLT-C List A Trial 5	CVLT-II List A Trial 5
	Recognition	CVLT-C Discriminability (age 5 only)	CVLT-C Discriminability	CVLT-II Discriminability
	Delayed Recall	CVLT-C Short Delay Free Recall (age 5 only)	CVLT-C Short Delay Free Recall	CVLT-II Short Delay Free Recall
		CVLT-C Long Delay Free Recall (age 5 only)	CVLT-C Long Delay Free Recall	CVLT-II Long Delay Free Recall
EMOTIONAL/ BEHAVIORAL FUNCTIONING		Child Behavior Checklist Behavior Problems		
	Internalizing Behavior Problems	CBCL Internalizing Problems	CBCL Internalizing Problems	NA
	Externalizing Behavior Problems	CBCL Externalizing Problems	CBCL Externalizing Problems	NA

[a]*WPPSI-III* Wechsler Preschool and Primary Scales of Intelligence third edition.
[b]*IQ* Intelligence Quotient.
[c]*WASI* Wechsler Abbreviated Scales of Intelligence, 4-Subtests.
[d]*Beery* Beery-Buktenica Developmental Test.
[e]*ADHD* Attention Deficit Hyperactivity Disorder.
[f]*BRIEF* Behavior Rating Inventory of Executive Function.
[g]*BRIEF-P* Behavior Rating Inventory of Executive Function – Preschool ages 2–5.
[h]*BRIEF-2* Behavior Rating Inventory of Executive Function 2nd Edition ages 5–18 years.
[i]*BRIEF-A* Behavior Rating Inventory of Executive Function-Adult version.
[k]*CVLT-II* California Verbal Learning Test – Second Edition.

in score based on p-values (0.05) were identified. In the 2. step those biomarker variables were investigated for an association with significant change in neuropsychological test score. For both steps quantile regression was used. This is analogous to linear regression in that it estimates a line through the data that minimizes the absolute (rather than squared) residuals. Quantile (median) regression analysis in Stata 16[9,10] estimates the size and statistical significance of the median change in paired subject scores between two NPAs +/− 95% confidence interval (CI) while relaxing the normality assumption and reducing the effect of extreme values.

For the tests showing significant change, quantile (median) regression models were implemented in step 2 to assess the strength and statistical

Table 2. Age at enrollment and at baseline neuropsychological assessment as well as age at next age-specific follow-up neuropsychological assessment.

Age at Enrollment (yrs)	Neuropsychological Test Battery at Baseline (yrs)	Next Follow-up Neuropsychological Testing Battery (yrs)
3–4	4	8
5	5	8
6	6	8
7–13	8	15
14–16	15	18
17–99	18	N/A, only after HA episode with coma

yrs years, *N/A* not applicable.

significance of the association between biomarker variables and the magnitude of change. As in step 1 and analogous to linear regression, these models estimated the median change +/− 95% CIs with the p-value expressing the probability that a difference as large or larger could be attributed to chance. These regression models, in each of the steps, controlled for differences in sex, initial test score and age at the beginning of the analysis period, as well as length of the interval between NPAs. Statistical significance was defined as a p-value of < 0.05.

RESULTS

Study population

The characteristics of the LS participants contributing data to this study are shown in Supplementary Table 1. There were 203 subjects who did not receive liver transplantation and who had two NPAs and an additional 17 subjects who had 2 NPAs prior to receiving liver transplantation for a total of 220 subjects, 74 of these participants had either 2 Bayley scales of infant development or one Bayley and one assessment at 3 y of age or older so only 146 of the 220 participants had 2 comparable NPAs at 3 y of age or older and were included in this analysis. The age ranges of these 146 participants at first and follow-up assessment are also shown in Supplementary Table 1.

Neuropsychological test performances

We started with determining the performance on neuropsychological tests at the first assessment and then assessed which neuropsychological tests showed statistically significant changes in test scores between the first and the second assessments (Table 3a). Table 3b displays how many participants with FSIQ < 70, how many participants with FSIQ ≥ 70, and how many participants with no FSIQ score available provided results for the respective tests. There were only 11 participants (13 when counting those with at least one FSIQ) with FSIQ < 70; instead of receiving the more limited assessment described in the method section, there was one participant in that group that had the CVLT and the Beery VMI administered and another one that had the Beery VMI administered, while only one received motor testing, and in about half of the participants with FSIQ < 70 parent questionnaires were completed; 59 participants (63 when counting those with at least one FSIQ) had a FSIQ ≥ 70, and for 70 participants no FSIQ was available. In the group of 70 participants with missing FSIQ, 24 participants had 2 Beery VMI assessments, 12 of those 24 had either an ABAS General Adaptive Composite (GAC) or a PIQ with scores above 70, indicating better functioning. Table 3b shows that the majority of tests (at a minimum two thirds of the total number of assessments performed for each neuropsychological test) were conducted in the group of participants with FSIQ ≥ 70.

Neuropsychological tests with significant decrease in test scores between two assessments

Table 3a shows that statistically significant differences in standardized, rescaled test scores between two assessments were only seen in the Visual Skills domain with a decrease in the median Beery VMI score (−5 decrease; $p = 0.05$) reflecting the ability to copy increasingly complex geometric figures, and in the Memory domain with a decrease in the median CVLT List A Trial 5 (involving recall of a list of words presented 5 times) and the median CVLT Discriminability score (involving recognition of words presented after a 20 min delay; −7.5 decrease; $p = 0.02$ for both). Median score changes corresponded to one-third to one-half of a standard deviation. Dominant hand grip strength showed an almost one-half of a standard deviation decrease in median score which was not significant (−6.7 decrease in score; $p = 0.17$). Performance on those tests was then related to ammonia and glutamine biomarker variables assessed in the time period between the two NPAs, to determine if they were associated with the score change.

Association of decline in neuropsychological test scores with biomarkers for UCD

We assessed the length of the interval and the metabolic control between 2 NPAs in study participants. As shown in Table 4, the median interval between 2 assessments differed by UCD diagnosis group; the interval between assessments was longest in asymptomatic individuals with OTCD. As expected, based on the biochemical defect, median ammonia and glutamine levels outside of HAE were highest in the proximal UCDs group. Notably, peak ammonia levels during HAE were not significantly different between proximal and distal UCDs although presenting glutamine levels were higher in the proximal disorders.

To investigate the relationship between metabolic status during the time of study and longitudinal changes in neuropsychological outcome, we used quantile regression analysis to relate metabolic biomarker variables to the median change in neuropsychological test scores. For this analysis we used those neuropsychological test scores that changed significantly between two assessments, i.e., the Beery VMI, CVLT List A Trial 5, and CVLT Discriminability scores. The CVLT List A Trial 5 sub-test is a measure of immediate recall and encoding. The CVLT List A Trials 1–5 Total score also assesses immediate recall and encoding[11] and is considered to be a measure of learning efficiency[12] as such, this sub-test was also included in the analysis. The analysis controlled for differences in sex, the initial test score, length of interval between NPAs, and age at the beginning of the analysis period. The relationship between each biomarker and interval change in neuropsychological test score is shown in Table 5 (test scores were rescaled to allow comparison).

We found that a decrease in the Beery VMI and the CVLT List A Trial 5 score was significantly associated with having experienced a HAE (Beery VMI median score decrease 0.4 SD, $p = 0.034$, CVLT List A Trial 5 median score decrease 1.4 SD, $p = 0.011$), and with having experienced 1–3 or 4 or more HAEs (Beery VMI median score decreased 0.4 SD for both, $p = 0.054$, CVLT List A trial 5 median score decreased 1.4 and 1.3 SD respectively, $p = 0.043$). Having a median peak ammonia level ≥300 µmol/L during HAE was associated with a Beery VMI median score decrease of 0.4 SD ($p = 0.058$) and a CVLT List A Trial 5 median score decrease of 1.1 SD ($p < 0.001$). There was no significant association between glutamine ≥1200 µmol/L during HAEs and Beery VMI and CVLT scores, although there was a generally consistent pattern of lower Beery VMI and CVLT List A Trial 5 and Discriminability scores with Gln ≥1200 µmol/L during HAEs.

Outside of HAEs, having median ammonia levels of 50 µmol/L and higher and 70 µmol/L and higher during long-term treatment was significantly associated with decrease in Beery VMI score (median score decrease 0.3 SD, $p = 0.029$ and 0.018, respectively). There was no statistically significant evidence of an association

Table 3. (a) First and second neuropsychological assessment scores and paired differences of scores between first and second assessment. (b) Neuropsychological Test Results at first assessment for participants with IQ less than 70, equal to or over 70, or not available.

a

Neuropyschological Assessment	N	First Assessment			Second Assessment			Paired Difference Over Interval			
		Median	min	max	Median	min	max	Median	min	max	*p*-value
Full Scale IQ[a]	70	94.0	53.0	136.0	92.0	50.0	129.0	−1.0	−26.0	27.0	0.520
ABAS GAC[b]	60	81.5	40.0	120.0	82.0	40.0	120.0	0.0	−42.0	45.0	1.000
Verbal IQ	69	100.0	55.0	134.0	97.0	55.0	133.0	1.0	−27.0	26.0	0.580
Performance IQ	74	88.5	53.0	132.0	88.5	46.0	126.0	0.0	−24.0	40.0	1.000
Beery[c] Visual Motor Integr.	75	87.0	48.0	123.0	87.0	45.0	115.0	**−5.0**	−26.0	40.0	**0.050**
Beery Visual Perception	72	89.5	45.0	137.0	92.0	45.0	124.0	1.0	−50.0	45.0	0.643
Grooved Pegboard (Dom)	69	91.4	40.0	117.3	93.4	40.0	121.7	0.4	−49.7	68.5	0.850
Grooved Pegboard (Nondom)	67	89.8	40.0	113.6	91.0	40.0	120.0	−0.2	−60.4	60.7	0.948
Grip Strength (Dom)	53	88.9	40.0	132.9	79.2	40.0	131.0	**−6.7**	−69.6	58.1	**0.170**
Grip Strength (Nondom)	52	87.3	40.0	138.2	81.6	40.0	131.1	**−4.3**	−45.1	41.2	**0.290**
CBCL[d] Attention	52	110.5	100.0	140.5	113.5	98.5	149.5	0.0	−15.0	40.5	1.000
TEA-Ch[e] Score!	13	85.0	70.0	125.0	90.0	70.0	110.0	0.0	−30.0	40.0	1.000
Teach Walk Don't Walk	12	77.5	55.0	125.0	80.0	55.0	125.0	0.0	−45.0	35.0	1.000
Brief[f] Shift	54	105.3	79.0	145.0	104.5	82.0	161.5	1.5	−37.5	46.5	0.641
Tower of London Move	18	80.0	60.0	114.0	74.0	60.0	122.0	−2.0	−50.0	50.0	0.836
CVLT[g] List A Total 1–5	43	101.5	34.5	146.5	94.0	64.0	131.5	−1.5	−36.0	85.1	0.718
CVLT List A Trial 1	43	100.0	62.5	160.0	100.0	62.5	137.5	0.0	−52.5	45.0	1.000
CVLT List A Trial 5	43	100.0	55.0	122.5	92.5	47.5	130.0	**−7.5**	−45.0	45.0	**0.020**
CVLT Discriminability	39	100.0	25.0	137.5	92.5	25.0	130.0	**−7.5**	−60.0	60.0	**0.020**
CVLT Short Delay Recall	43	100.0	55.0	122.5	100.0	62.5	160.0	0.0	−30.0	75.0	1.000
CVLT Long Delay Recall	42	100.0	62.5	130.0	100.0	47.5	130.0	0.0	−45.0	52.5	1.000
Brief Working Memory	54	118.0	82.0	157.0	116.5	83.5	163.0	−3.0	−36.0	48.0	0.352
CBCL Internalizing Behavior	47	104.5	74.5	131.5	109.0	74.5	137.5	3.0	−36.0	42.0	0.228
CBCL Externalizing Behavior	47	101.5	73.0	140.5	106.8	76.0	137.5	−1.5	−40.5	33.0	0.702

b

Neuropyschological Assessment	N	Full Scale IQ<70[1]			N	Full Scale IQ≥70[2]			N	No Full Scale IQ			Total
		median	min	max		median	min	max		median	min	max	N
Full Scale IQ[a]	11	61.0	53.0	67.0	59	98.0	71.0	136.0	70	.	.	.	140
ABAS GAC[b]	6	48.0	43.0	57.0	35	90.0	54.0	120.0	19	74.0	40.0	120.0	60
Verbal IQ	11	55.0	55.0	78.0	57	104.0	72.0	134.0	1	58.0	58.0	58.0	69
Performance IQ	11	61.0	53.0	79.0	56	93.0	60.0	132.0	7	75.0	59.0	96.0	74
Beery[c] Visual Motor Integration	2	56.0	48.0	64.0	49	89.0	49.0	123.0	24	82.5	58.0	108.0	75
Beery Visual Perception	1	45.0	45.0	45.0	47	88.0	48.0	137.0	24	94.0	56.0	131.0	72
Grooved Pegboard (Dominant)	0	.	.	.	47	88.5	40.0	115.9	22	92.2	40.0	117.3	69
Grooved Pegboard (Nondom)	0	.	.	.	45	89.0	40.0	112.8	22	91.6	40.0	113.6	67
Grip Strength (Dominant)	1	74.2	74.2	74.2	34	83.1	40.0	110.1	18	103.5	40.0	132.9	53
Grip Strength (Nondominant)	1	75.4	75.4	75.4	33	82.2	40.0	121.6	18	105.0	40.0	138.2	52
CBCL[d] Attention	1	130.0	130.0	130.0	35	113.5	100.0	140.5	16	106.0	100.0	134.5	52
TEA-Ch[e] Score!	0	.	.	.	10	82.5	70.0	125.0	3	105.0	75.0	110.0	13
Tea-Ch Walk Don't Walk	0	.	.	.	9	70.0	55.0	125.0	3	110.0	55.0	120.0	12
Brief[f] Shift	4	111.3	109.0	130.0	40	101.5	79.0	145.0	10	105.3	82.0	140.5	54
Tower of London Move	0	.	.	.	15	80.0	60.0	114.0	3	76.0	74.0	86.0	18

Table 3. continued

b

Neuropyschological Assessment	N	Full Scale IQ<70[1]			N	Full Scale IQ≥70[2]			N	No Full Scale IQ			Total
		median	min	max		median	min	max		median	min	max	N
CVLT[g] List A Total 1-5	1	61.0	61.0	61.0	40	101.5	34.5	146.5	2	104.5	83.5	125.5	43
CVLT List A Trial 1	1	62.5	62.5	62.5	40	100.0	62.5	160.0	2	111.3	100.0	122.5	43
CVLT List A Trial 5	1	92.5	92.5	92.5	40	100.0	55.0	122.5	2	103.8	92.5	115.0	43
CVLT Discriminability	0	.	.	.	37	100.0	25.0	137.5	2	100.0	92.5	107.5	39
CVLT Short Delay Recall	1	77.5	77.5	77.5	40	103.8	55.0	122.5	2	103.8	85.0	122.5	43
CVLT Long Delay Recall	1	85.0	85.0	85.0	39	100.0	62.5	130.0	2	103.8	85.0	122.5	42
Brief Working Memory	4	125.5	119.5	130.0	40	117.3	82.0	157.0	10	115.8	100.0	140.5	54
CBCL Internalizing Behavior	0	.	.	.	31	100.0	74.5	131.5	16	109.8	88.0	131.5	47
CBCL Externalizing Behavior	0	.	.	.	31	104.5	73.0	140.5	16	101.5	80.5	125.5	47

Bold values denote the important findings described in the results section.
[a] *IQ* Intelligence Quotient.
[b] *ABAS GAC* Adaptive Behavior Assessment System General Adaptive Composite.
[c] *Beery* Beery-Buktenica Developmental Test.
[d] *CBCL* Child Behavior Checklist.
[e] *Tea-Ch* Test of Everyday Attention for Children.
[f] *BRIEF* Behavior Rating Inventory of Executive Function.
[g] *CVLT* California Verbal Learning Test.
[1] *N 13* when counting those with at least one FSIQ.
[2] *N 63* when counting those with at least one FSIQ.

between median Glutamine ≥800 µmol/L or ≥1000 µmol/L and a change in Beery VMI or CVLT test scores.

DISCUSSION

As cognitive impairment is often reported in UCD,[13] neuropsychological function is one of the most important clinical outcomes for patients with UCDs and therefore a focus of the UCDC's LS. The objective of our study was to determine whether neuropsychological functioning declines during the long-term treatment of UCD and, if so, whether there is an association between biomarkers of metabolic control and biomarkers of outcome (performance on neuropsychological tests). Our hypothesis was that elevated ammonia and/or glutamine levels in patients with UCD are associated with neuropsychological decline.

We found significant declines on measures of visual-motor integration and memory encoding (Beery VMI, CVLT List A Trial 5, CVLT Discriminability) between 2 NPAs (Table 3a). After determining which neuropsychological tests showed significant decline in scores during long-term treatment, we assessed whether these changes were associated with the biomarkers of metabolic control we had defined a priori. Decline in measures of visual motor integration and immediate-recall, memory encoding (Beery VMI and CVLT List A Trial 5) was significantly associated with experiencing any HAE and with increased frequency of HAEs. There was also a significant decrease in a measure of immediate-recall, memory encoding (CVLT List A Trial 5 score) with median peak ammonia levels ≥300 µmol/L during HAEs. There was no significant association between high glutamine levels during HAEs and subsequent decline in neuropsychological test scores. During long-term treatment, having median ammonia levels above the normal range was associated with a significant decrease in the visual-motor integration (Beery VMI) score; however, glutamine ≥1000 µmol/L (the level at which management changes are usually implemented to prevent HAE) was not significantly associated with a change in neuropsychological test scores over time. These results indicate that elevated ammonia levels, and HAEs, are associated with decreased performance in visual motor integration and immediate-recall, memory encoding. This suggests that, associated with these ammonia variables, patients experience increasing difficulties with learning new information presented verbally (CVLT) and increasing difficulties with using visual information to direct their motor movements (Beery VMI).[14]

The CVLT assesses encoding / storing of new information in memory, retrieval of information from memory, as well as learning strategies.[11,12] We saw a significant decrease in the CVLT List A Trial 5 and the CVLT Discriminability score between two assessments.

While CVLT List A Trial 5 is a measure of immediate-recall and encoding,[11] performance on this test is also thought to be affected by working memory[15] and sustained attention.[11] The Discriminability score on the other hand assesses encoding by measuring recognition.[11] Although we did not see a significant decrease in another measure of recall and encoding, the List A Trials 1–5 Total score, the fact that both, the List A Trial 5 and the Discriminability scores decreased suggests that patients with UCD have encoding problems when it comes to verbal learning. This result may be useful in developing interventions directed at improving encoding problems in patients with UCD.

Previous research identified several affected neuropsychological domains in UCDs, including intellectual function, executive function, and motor skills. Gropman et al.[16] showed evidence that working memory and executive function is affected in OTCD. We did not see a significant change in scores on tests from the attention/executive domain including BRIEF working memory from one NPA to the next. This may reflect that the BRIEF is a parent-report measure for children. Sprouse et al.[2] reported that executive function, motor ability and in a small group of symptomatic OTCD patients also visual motor integration (VMI) was significantly affected. This was a first indication that VMI is affected in UCD. Waisbren et al.[17] related available lifetime biomarker levels to latest neuropsychological outcome, i.e., they

Table 4. Metabolic control in the interim between first and second neurpsychological assessment by urea cycle disorder (UCD) diagnosis group.

Characteristics Of Study Interval	N	Mean	sd	Proximal UCD[b]		max	N	Mean	sd	Asymptomatic OTCD[c]			N	Mean	sd	Distal UCD		max
				Median	min					Median	min	max				Median	min	
Duration of Interval (years)	98	4.75	2.69	4.2	0.3	11.21	22	5.96	2.11	6.43	1.42	9.11	100	4.67	2.91	4	0.06	10.81
Interval Levels outside of HAE[a]																		
Ammonia Level	97	36.78	20.44	36	8	149	22	23.41	10.3	25	9	41	99	31.31	18.65	28.5	8.7	113
Glutamine Level	97	842.4	185.7	854	418	1331	22	689.8	134.4	664	544	1023	100	618.7	112.7	604	380	927
Interval Levels During HAE																		
Peak Ammonia Level	38	262.6	182	215	119	1106	0						30	324.1	230.1	298	34	977
Presenting Glutamine Level	20	842.4	185.7	854	418	1331	0						18	918.6	343.3	901	290	1784

[a] *HAE* Hyperammonemic Event.
[b] *UCD* Urea Cycle Disorder.
[c] *OTCD* Ornithine transcarbamylase deficiency.

assessed the most recent status of a patient but not change over time. They reported that study participants with distal UCD (ASD, ALD, ARGD) ages 3 years and older had FSIQ scores in the ID (ALD and ARGD) or borderline (ASD) range. In addition to FSIQ scores, they observed the poorest functioning in the Visual Performance and Motor domains which included visual motor integration (Beery VMI). HAE was associated with poorer performance in the visual motor performance domain, consistent with our findings.

Posset et al.[18] analyzed cross-sectional data from 503 individuals with UCD. These investigators examined UCD diagnosis, age at disease onset, delay of diagnosis, therapy, initial clinical presentation, and peak ammonia and glutamine level to determine how these variables affected neurocognitive outcome as measured by global function measures. They found that symptomatic individuals had a significantly lower cognitive score than asymptomatic ones and that patients with neonatal onset disease performed significantly worse than patients with late-onset disease. A high initial ammonia was significantly associated with intellectual disability. Buerger et al.[19] conducted a cross-sectional study of the first assessments in LS participants with OTCD greater than 3 years of age and determined that intercorrelations between cognitive domains were high. Their conclusion was that 'OTCD has a global impact on cognitive functioning rather than a specific effect on distinct cognitive domains'. In contrast our study showed that the memory domain and the visual skills domain are specifically affected in UCD.

In conclusion, our study supports earlier findings that a UCD negatively affects visual motor skills (VMI).[2,17] In addition, our study showed that the VMI score decreases over time and that this decrease is associated with biomarkers of metabolic control. A decline in verbal learning over time, that may be due to impairment in encoding, is a new finding in UCD that was not reported previously. It is possible that the decline in scores over time does not represent a true decline but rather a slower gain in encoding and visual motor integration skills compared to the norm and that this slowing of the developmental trajectory results in the gap between patients and typically developing individuals to widen. In this scenario, the slower gain and wider gap is associated with the ammonia variables we identified. Whether there is slower gain or decline over time, the Beery VMI and CVLT List A Trial 5 score may be important biomarkers of outcome for clinical trials investigating new treatments for UCD. However, it will be important to keep in mind what the target population of the trial is as these instruments were not sufficiently assessed in patients with an IQ of less than 70.

Our study failed to find worsening performance in the Attention/Executive Function domain over time, while prior publications reported that mildly symptomatic and asymptomatic, high-functioning females who are heterozygous for a pathogenic variant in *OTC* showed weaknesses in fine motor dexterity/speed (significant finding) and in nonverbal intelligence, visual memory, attention/executive skills, and math (non-significant finding).[20] Furthermore, deficits in the Attention/Executive Function domain, especially attention problems,[5] working memory deficits,[16,21] and poorer performance on the Plan/Organize and Initiate subscales of the BRIEF[21] were previously demonstrated in individuals with UCDs. With regard to change over time, Diaz et al.[22] reported improvement of BRIEF scores, including working memory, with improved metabolic control.

Although our clinical experience is consistent with the reported findings of deficits in the Attention/Executive Function domain in UCD our analysis failed to detect a significant decline of scores in this domain over time. Specifically, we did not see a significant decline in the BRIEF working memory score over time. It is possible that the fact that the BRIEF was only administered to very few (4) participants with FSIQ < 70 and to 10 with no-IQ score limited our ability to detect change in this domain. This would be the case if the working memory of participants in these two groups would be most affected by metabolic insults. However, regarding change in

Table 5. Relationship between change in neuropsychological test scores (standardized to mean = 100, sd = 15) between two assessments and metabolic control during the interval between assessments, controlled for: initial score, sex, age at testing, duration between assessments.

Biomarkers of Metabolic Control	N	Beery VMI[a]		CVLT[b] List A Trial 5			CVLT List A Total 1–5			CVLT Discriminability		
		Median	95% CI	N	Median	95% CI	N	Median	95% CI	N	Median	95% CI
Hyperammonemic Events (HAEs)												
No	49	2.1	[−3.3, 7.5]	33	−4.5	[−13.2, 4.2]	33	−4	[−15.0, 6.9]	30	−3.4	[−11.4, 4.5]
Yes	26	−5.5	[−9.9, −1.1]	10	−20.3	[−31.7, −8.9]	10	−10.8	[−22.0,0.5]	9	−0.7	[−27.4, 26.0]
P-value		0.034			0.011			0.275			0.845	
Biomarkers Associated with HAEs												
Frequency of HAEs												
None	49	1.9	[−3.4, 7.5]	33	−5.0	[−13.8, 3.9]	33	−4.0	[−15.1, 7.1]	30	−3.6	[−11.4, 4.2]
1–3	18	−5.4	[−15.1, 4.3]	4	−20.7	[−34.0, −7.5]	4	3.1	[−10.0,16.0]	3	15.5	[−23.1, 54.0]
4 +	8	−5.7	[−10.7, −0.7]	6	−19.3	[−31.9, −6.7]	6	−11.5	[−26.6, 3.5]	6	−23.0	[−55.3, 9.2]
P-value		0.054			0.043			0.230			0.338	
Peak Ammonia Levels of HAEs												
No HAEs	49	1.3	[−4.2, 6.9]	33	−4.5	[−13.2, 4.2]	33	−4.0	[−15.2, 7.2]	30	−3.8	[−11.4, 3.9]
Median Peak Ammonia < 300	17	−4.7	[−15.0, 5.6]	7	−24.6	[−35.3, 13.8]	7	−10.6	[−24.3, 3.0]	6	−14.2	[−47.8, 19.4]
Median Peak Ammonia ≥ 300	9	−6.0	[−11.7, −0.2]	3	−16.8	[−27.9, −5.8]	3	−11.1	[−24.5, 2.2]	3	6	[−2.6, 14.6]
P-value		0.058			<0.001			0.587			0.185	
Cumulative Peak Ammonia Levels of HAEs												
No HAEs	49	1.9	[−3.7, 7.4]	33	−6.5	[−15.4, 2.4]	33	−4.1	[−15.6, 7.3]	30	−2.3	[−10.2, 5.6]
100–440	11	−6.3	[−11.7, −0.8]	2	−26.8	[−130.6, 77.1]	2	−0.4	[−94.7, 93.9]	1	57.7	[49.8, 65.6]
441 +	15	−3.9	[−11.2, 3.4]	8	−13.1	[−25.7, −0.4]	8	−10.8	[−25.3, 3.7]	8	−17.3	[−42.2, 7.6]
P-value		0.100			0.492			0.722			0.258	
Peak Glutamine Levels of HAEs												
No HAEs	49	1.6	[−4.4, 7.5]	33	−5.1	[−13.8, 3.5]	33	−4.0	[−15.3, 7.3]	30	−3.4	[−11.2, 4.4]
Median Peak Glutamine < *1200*	18	−3.3	[−12.3, 5.8]	6	−10.5	[−24.9, 3.8]	6	−10.8	[−23.6, 2.1]	5	−0.7	[−20.3, 18.9]
Median Peak Glutamine ≥ *1200*	8	−7.1	[−13.3, −0.8]	4	−24.6	[−46.0, 3.3]	4	0.1	[−29.2, 29.4]	4	−28.5	[−95.1, 38.1
P-value		0.118			0.260			0.502			0.733	
Biomarkers Outside of HAEs												
Median Ammonia Level												
Median Ammonia Level < 70	45	2.7	[−3.0, 8.4]	31	−7.5	[−17.3, 2.2]	31	−2.3	[−14.8, 10.1]	28	−4.9	[−15.1, 5.4]
Median Ammonia Level ≥ 70	30	−4.8	[−8.6, −1.0]	12	−12.8	[−27.9, 2.3]	12	−7.3	[−25.8, 11.2]	11	6.3	[−13.5, 26.2
P-value		0.018			0.581			0.689			0.321	
Median Ammonia Level < 50	31	3.5	[−3.6, 10.6]	24	−7.2	[−17.2, 2.7]	24	2.6	[−11.2, 16.4]	22	−3.7	[−14.4, 7.0]
Median Ammonia Level ≥ 50	44	−4.7	[−8.8, −0.6]	19	−11.0	[−21.6, −0.43]	19	−9.5	[−19.8, 0.9]	17	−7.4	[−21.6, 6.9]
P-value		0.029			0.610			0.148			0.663	
Median Glutamine Level												
Median Glutamine Level < 1000	40	−4.5	[−9.3, 0.3]	26	−6.9	[−15.7, 1.8]	26	−2.7	[−16.2, 10.8]	25	2.6	[−7.7, −12.9]
Median Glutamine Level ≥ 1000	35	−2.7	[−8.9, 3.6]	17	−12.6	[−25.3, 0.2]	17	−6.9	[−15.9, 2.2]	14	−6.9	[−22.5, 8.7]

Table 5. continued

Biomarkers of Metabolic Control	N	Beery VMI[a]		CVLT[b] List A Trial 5			CVLT List A Total 1–5			CVLT Discriminability		
		Median	95% CI	N	Median	95% CI	N	Median	95% CI	N	Median	95% CI
P-value		0.642			0.481			0.592			0.323	
Median Glutamine Level < 800	26	−5.2	[−13.2, 2.8]	21	−8.3	[−15.7, −0.9]	21	−2.7	[−15.3, 9.9]	20	−7.9	[−20.8, 4.9]
Median Glutamine Level ≥ 800	49	−2.5	[−8.0, 3.1]	22	−9.2	[−23.2, 4.8]	22	−7.3	[−19.4, 4.8]	19	1.4	[−10.4, 13.3]
P-value		0.592			0.909			0.598			0.214	

[a]*Beery VMI* Beery-Buktenica Developmental Test of Visual Motor Integration.
[b] *CVLT* California Verbal Learning Test.

test performance for participants with FSIQ < 70 in general we hypothesize that participants with a severe initial insult from hyperammonemic coma may be showing less change over time because the initial injury has such an overwhelming effect on neurocognitive functioning that this impacts our ability to detect subtler subsequent changes, similar to moderate to severe traumatic brain injury where features of the injury explain some of the long-term differences in test performance.[15] However, children with TBI do not experience ongoing harm to the brain from metabolic insults as children with UCD do so one cannot directly compare the two groups. Overall, our ability to detect significant change over time for more than two of the tests administered was certainly impacted by the number of participants for whom two assessments per test were available. However, two assessments were available for other tests from a similar number of participants as for the CVLT and the Beery VMI. Our ability to detect significant change over time in any additional neuropsychological tests performed may have been impacted by the number of participants with FSIQ < 70 that were tested. However, we do not know if these patients are more or less likely to experience further decline due to metabolic insults and if their test results would have impacted our overall results. In our study, the CVLT and the Beery VMI appear more sensitive for detecting effects of metabolic challenges occurring in participants with FSIQ ≥ 70 than other neuropsychological tests used.

A shortcoming of our study is that biomarkers of metabolic control were only assessed every 6 months in children and adolescents and annually in adults. We thus did not have a continuum of metabolic biomarker levels but rather intermittent levels for our analysis, nevertheless we were able to find an association between biomarkers of metabolic control and outcome. Also, a larger number of patients with 2 NPAs per age group would be desirable. Another consideration is the fact that we were looking at blood glutamine levels and Pacheco-Colon et al.[23] reported that brain glutamine levels can substantially differ from blood glutamine levels with brain glutamine levels being potentially elevated in the absence of elevated blood glutamine.

Finally, it has long been established that global intellectual dysfunction and ID occur in UCDs[1,5,6] and that high ammonia levels are damaging to the brain.[1] The damaging effects of ammonia were further underscored by the fact that developmental disabilities in individuals with UCDs were associated with the number and the duration of hyperammonemic episodes experienced by patients.[5,17,19,24] Our study did not find significant worsening of global function during long-term treatment rather decline or slower progression in two specific neuropsychological functions over time and decreased performance was associated with ammonia biomarkers. We demonstrated that visual motor integration and immediate recall, memory encoding are specifically affected by hyperammonemic crises and visual motor integration also by long-term elevated ammonia levels above the normal range. The very important task for the treating physician is thus the prevention of hyperammonemic crises and of elevated ammonia levels during long-term treatment. Elevated glutamine levels were previously shown to be harbingers of hyperammonemic crises[3,25] indicating high nitrogen load. They are therefore valuable clinical parameters that indicate to the treating physician when treatment adjustments need to be made to prevent elevted ammonia levels and a hyperammonemic crisis. Glutamine levels are also less prone to artifacts of sample handling and show less variability depending on the time of day.[25] They are thus important biomarkers of metabolic control for treatment monitoring in the out-patient clinic.

DATA AVAILABILITY

As per the policies of the NIH RDCRN, all data from a particular consortium will be deposited into dbGaP. This data deposition will be done after the funding period for

the consortium is completed. At the current time, we are not able to submit participant-level data in a public repository.

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AUTHOR CONTRIBUTIONS

U.L.-K., J.H.S., and R.C. substantially contributed to conception and design, acquisition of data, data analysis and drafting of the article as well as revising it critically for important intellectual content. All three authors provided final approval of the version to be published. The members of the UCDC contributed to acquisition of data and revising the manuscript critically and provided final approval of the version to be published.

COMPETING INTERESTS

U.L.-K., J.H.S., and R.C. have no potential conflicts of interest regarding this study. Of the consortium authors: Mathias Baumgartner received a research fund from Nutricia and is a member of the clinical advisory boards of Hemoshear and Moderna neither of these involvements is regarding Urea Cycle disorders. George Diaz is now working for iECure. His involvement in the UCDC occurred, while he was employed by Icahn School of Medicine at Mount Sinai and he declared no competing interests regarding this manuscript. Gregory Enns receives compensation as a consultant for AllStripes, Hemoshear, Horizon Therapeutics, M6P Therapeutics, and Ultragenyx Pharmaceutical and clinical trial support from Aeglea Biotherapeutics. J Lawrence Merritt currently reports employment by, and stock ownership in, Ultragenyx Pharmaceutical Inc. His involvement in the UCDC occurred, while JLM was employed by Seattle Children's Hospital, prior to this Ultragenyx employment and stock ownership. Andreas Schulze is consulting or on the advisory board of Ultragenyx and Horizon and conducts industry initiated clinical studies for Aeglea. Susan Waisbren consults Ultragenyx Gene Therapy.

CONSENT STATEMENT

The Longitudinal Study of the UCDC was approved by the IRBs at all participating sites and informed consent/assent was obtained from all study participants or legal guardians.

ADDITIONAL INFORMATION

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