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ORIGINAL ARTICLE

Biochemical markers and neuropsychological functioning in distal urea cycle disorders

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Abstract

Urea cycle disorders often present as devastating metabolic conditions, resulting in high mortality and significant neuropsychological damage, despite treatment. The Urea Cycle Disorders Longitudinal Study is a natural history study that collects data from regular clinical follow-up and neuropsychological testing. This report examines links between biochemical markers (ammonia, glutamine, arginine, citrulline) and primary neuropsychological endpoints in three distal disorders, argininosuccinic acid synthetase deficiency (ASD or citrullinemia type I), argininosuccinic acid lyase deficiency (ASA or ALD), and arginase deficiency (ARGD). Laboratory results and test scores from neuropsychological evaluations were assessed in 145 study participants, ages 3 years and older, with ASD ($n = 64$), ASA ($n = 65$) and ARGD ($n = 16$). Mean full scale IQ was below the population mean of 100 ± 15 for all groups: (ASD = 79 ± 24; ASA = 71 ± 21; ARGD = 65 ± 19). The greatest deficits were noted in visual performance and motor skills for all groups. While ammonia levels remain prominent as prognostic biomarkers, other biomarkers may be equally valuable as correlates of neuropsychological functioning. Cumulative exposure to the biomarkers included in the study proved to be highly sensitive indicators of neuropsychological outcomes, even when below the cut-off levels generally considered toxic. Blood levels of biomarkers obtained on the day of neuropsychological evaluations were not correlated with measures of functioning for any disorder in any domain. The importance of cumulative exposure supports early identification and confirms the need for well-controlled management of all biochemical abnormalities (and not just ammonia) that occur in urea cycle disorders.

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Introduction

The urea cycle is responsible for the detoxification of ammonia (NH_4^+) to urea. When enzymes responsible for the sequential steps in the urea cycle are absent or deficient, ammonia builds up in the brain and other tissues (Batshaw and Brusilow 1980; Kölker et al 2015). Ammonia accumulation or increases in glutamine (a consequence of hyperammonemia) lead to astrocyte swelling and subsequent damage. Depending on the age of onset and duration and severity of the hyperammonemic episode, brain effects are temporary or permanent and can be mild or devastating (Enns 2008). Other mechanisms of injury may also operate. Treatment varies somewhat depending on the specific location of the enzyme block in the cycle. The basic elements of treatment are dietary protein restriction and ammonia scavenger drugs. Supplemental treatment with citrulline or arginine is required for most of the disorders. Genetic

defects in the urea cycle are generally categorized into proximal (mitochondrial) or distal (cytosolic) disorders, plus several transport disorders, which are known by the specific enzyme deficiency or biochemical abnormality.

The Urea Cycle Disorders Consortium (UCDC) is part of the Rare Diseases Clinical Research Network (RDCRN) and is supported jointly by the National Center for Advancing Translational Science, Office of Rare Diseases Research and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (U54HD061221). The goals of the RDCRN are to perform natural history studies of rare diseases and to hasten the bringing to market of orphan products to treat rare disorders (Batshaw et al 2014; Seminara et al 2010). Philanthropic support supplements these research activities, as well. The UCDC Longitudinal Study, now in its 11th year, collects medical and neuropsychological information on the eight different urea cycle disorders. This paper focuses on three distal disorders, argininosuccinate synthetase deficiency (ASD or citrullinemia type I) (OMIM 215700), argininosuccinic acid lyase deficiency (ASA or ALD) (OMIM 207900), and arginase deficiency (ARGD) (OMM 207800), with the aim of characterizing the associations between biochemical markers (ammonia, glutamine, citrulline, and arginine) and neuropsychological outcomes. A better understanding of these biochemical markers and their relationships to specific functional domains has the potential to guide treatment and contribute to the design of clinical drug trials.

Methods

The Urea Cycle Disorders Longitudinal Study collects data from regular clinical follow-up as well as neuropsychological testing that in many centers would not have been part of routine care. The testing includes age appropriate measures of intelligence and global functioning, verbal abilities, visual performance, motor skills, and memory (Table 1). For this paper, we included results from children 3 years of age and older since we focused on neuropsychological outcomes that may not be measurable in younger children. Although some participants (35.2%) were evaluated more than once, we conducted analyses including only the most recent testing results in order to capture long-term risks. Evaluations were performed at a mean of 5.17 ± 5.10 years (median = 3.4, range = 0.1–23.6 years) after the last hyperammonemic episode. No one was evaluated while experiencing a hyperammonemic episode.

Biomarkers related to metabolic status included measurements of ammonia, glutamine, arginine, and citrulline. All

Table 1 Neuropsychological domains, sources of data, and variables

Domain	Source	Variables comprising the domain
Global Functioning	Wechsler Preschool and Primary Scales of Intelligence, Third Edition (WPPSI-III) (Wechsler 2002) or 4-Subtest Wechsler Abbreviated Intelligence Scale (WASI) (Wechsler, 1999 or 2011)	Full scale IQ
	Adaptive Behavior Assessment System, Second Edition (ABAS-II) (Harrison & Oakland 2003)	General Adaptive Composite (GAC)
	Behavior Rating Inventory of Executive Function (BRIEF)	Global Executive Composite (GEC)
	Achenbach Child Behavior Checklist (CBCL) (Achenbach & Rescorla 2000)	Total Problems Score
Verbal	WPPSI III or WASI	Verbal IQ
	Developmental NEuroPSYchological Assessment, Second Edition (NEPSY-II) (Korkman et al 2007) or Delas-Kaplan Executive Function System (D-KEFS) (Delis et al 2001)	Verbal fluency
	ABAS-II Communication Scale	Communication
Visual Performance	WPPSI III or WASI	Block Design*
	Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI) (Beery and Beery 2010)	Visual motor integration
Motor	Wide Range Assessment of Visual Motor Abilities (WRAVMA) (Adams and Sheslow 1995) or Grooved Pegboard (Lafayette Grooved Pegboard, Lafayette Instrument Co.)	Pegboard dominant hand
	WRAVMA or Lafayette Grooved Pegboard	Pegboard non-dominant hand
	Dynamometer (Lafayette Hand Dynamometer, Lafayette Instrument Co.)	Grip strength dominant hand
	Dynamometer	Grip strength non-dominant hand
Memory	California Verbal Learning Test (Child or Adult) (CVLT-C, CVLT) (Delis et al 1994; 2000)	List A trial 1 free recall List A trial 5 free recall
	Rey Osterreith Complex Figure (ROCF) (Meyers and Meyers 1995)	ROCF immediate recall
	Behavior Rating Inventory of Executive Function (BRIEF) (Roth et al 2005)	Working memory score

*This subtest also measures aspects of executive functioning

laboratories for this study were associated with the large medical centers affiliated with the Urea Cycle Consortium and were clinically certified. Certification included rigorous quality control procedures and proficiency testing. In all laboratories, the normal value of blood ammonia was consistently established at < 50 μmol/L. The arbitrary cut-off of 100 μmol/L to define a hyperammonemic episode was established based on past experience within the Urea Cycle Consortium. Data on laboratory values were obtained from a review of medical records. Parameters related to the biochemical values included the concurrent level on day of neuropsychological testing and mean lifetime exposure. If the participant had values that spanned 3 or more years, the mean within each year was first calculated. Then the mean and standard deviations were calculated from these values to obtain mean lifetime exposure. If the participant had values that spanned < 3 years, means and standard deviations were only calculated if the participant had one or more measurements. The number of hyperammonemic episodes (defined as ammonia levels above 100 μmol/L) and the number of ammonia "spikes" (defined as an ammonia level > 10 μmol/L above the mean for that individual) were also calculated. The degree of dispersion in laboratory values around the mean value for the individual was labeled the standard deviation score. Cumulative exposure was defined as the lifetime mean exposure X age up to and including the time of the neuropsychological evaluation. Older individuals thus have a higher cumulative exposure score. The standard deviation and cumulative exposure variables as defined above proved to be powerful predictors of outcome in a study of individuals with phenylketonuria (PKU) (Hood et al 2015). These parameters were included in our analyses because of the possibility that variability and persistence of abnormally elevated levels of biochemical toxins have a similar effect in urea cycle disorders as they do in PKU. In our study, for the individuals who had received a liver transplant to prevent hyperammonemia, cumulative exposure to ammonia was calculated up to the time of the liver transplant (rather than age at testing).

Associations between biomarkers and performance on each of the neuropsychological tests were determined through Pearson correlation analyses for each disorder separately. Scores from the separate tests measuring the same or similar neuropsychological function were converted to z-scores with a mean of 0 and a standard deviation of 1 to create a combined mean "domain" score. In order to simplify the tables, we report correlation coefficients (r) above $r = 0.30$. Analyses were computed using SAS. Data from male and female participants were combined since no gender differences were noted in the biomarkers or neuropsychological test results.

Table 2 Description of the sample (demographics and health history)

	ASD-citrullinemia	ASA	ARGD
Age at most recent evaluation (years) (n, mean ± SD, median, (range))	n = 64	*n* = 65	n = 16
	11.43± 7.25	14.87 ± 10.44	18.74 ± 11.20
	8.86	14.06	17.97
	(3.20–32.56)	(3.04–45.60)	(3.46–45.53)
Sex	Male: *n* = 29(45%)	Male: n = 29(45%)	Male: n = 5 (31%)
	Female: *n* = 35(55%)	Female: *n* = 36(55%)	Female: *n* = 11 (69%)
Age at diagnosis (years) (n, mean ± SD, median, (range))	n = 64	n = 65	n = 16
	1.13 ± 3.58	1.82 ± 3.29	4.5± 4.4
	0	0	3.5
	(0–21.00)	(0–14.00)	(0–13.00)
Neonatal/late onset	Neonatal: *n* = 17 (27%)	Neonatal: *n* = 15 (23%)	Neonatal: *n* = 0
	Late: *n* = 47 (73%)	Late: *n* = 50 (77%)	Late: n = 16 (100%)
Liver transplant	No: *n* = 51 (80%)	No: *n* = 54 (83%)	No: n = 16 (100%)
	Yes: *n* = 13 (20%)	Yes: *n* = 11 (17%)	Yes: n = 0
Method of identification *	Clinical: 43 (67%)	Clinical: *n* = 38 (59%)	Clinical: *n* = 14 (88%)
	Family: 5 (8%)	Family: *n* = 8 (12%)	Family: n = 2 (12%)
	NBS: 16 (25%)	NBS: *n* = 19 (29%)	NBS: n = 0
Age at time of evaluation for this study (years)	3–5: 16 (25%)	3–5: 14 (22%)	3–5: 2 (13%)
	6–16: 33 (52%)	6–16: 24 (37%)	6–16: 3 (19%)
	17+: 15 (23%)	17+: 27 (42%)	17+: 11 (69%)

n = number of participants; SD = standard deviation;

*Method of identification: Clinical = identified because of clinical symptoms; Family = identified because of an affected family member; NBS = identified through newborn screening

Table 3 Composite z-scores for neuropsychological domains and associated test scores (n, mean ± standard deviation, median, (range))

	ASD citrullinemia	ASA	ARGD
Global functioning domain	61	64	16
z-score	−0.75 ± 1.31	−1.29 ± 1.24	−1.37 ± 1.43
	−0.33	−1.03	−0.97
	(−3.67–2.60)	(−3.67–1.03)	(−3.33–0.69)
Full scale IQ	52	60	12
standard score	78.92 ± 24.06	71.47 ± 21.33	65.42 ± 18.79
normative mean =	77.5	69	60.5
100 ± 15	(43.0–133.0)	(50–141)	(50.0–111.0)
ABAS GAC	52	52	10
standard score	79.37 ± 23.55	75.58 ± 21.75	67.10 ± 26.11
normative mean=	83.5	73.5	57.5
100 ± 15	(40.0–117.0)	(40.0–127.0)	(40.0–116.0)
BRIEF GEC	30	28	6
scaled score	58.13± 10.52	59.79 ± 10.92	61.00 ± 16.53
normative mean = 50 ± 10	58	61.5	56
	(39–77)	(34.0–86.0)	(42–82)
CBCL total problems	42	31	10
scaled score	54.9± 10.1	53.29 ± 10.31	60.60 ± 9.98
normative mean = 50± 15	53.5	54.0	60
	(32.0–76.0)	(36.0–72.0)	(45–73)
Verbal domain	59	64	16
z-score	−1.03 ± 1.37	−1.55 ± 1.18	−1.88 ± 1.19
	−0.73	−1.57	−2.3
	(−3.10–1.60)	(−3.00–1.97)	(−3.0–0.3)
Verbal IQ	50	60	12
standard score	80.46 ± 23.24	74.9 ± 19.77	70.08 ± 18.74
normative mean =	80.5	71.5	61.5
100 ± 15	(52.0–143.0)	(55.0–139.0)	(55.0–107.0)
Verbal fluency	6	11	3
scaled score	6.17 ± 3.31	4.46 ± 2.91	7.33 ± 1.53
normative mean=	5.5	4	7
10 ± 15	(2–12)	(1–9)	(6–9)
ABAS communication	52	52	10
Normative scaled score	7.56 ± 4.46	6.3 ± 4.08	5.7 ± 4.62
mean=	9	6	4.5
10 ± 15	(1–14)	(1–16)	(1–12)
Visual motor performance domain	48	59	14
z-score	−1.39 ± 1.35	−1.91 ± 1.07	−2.19 ± 1.10
	−1.33	−2.33	−2.5
	(−3.00–2.00)	(−3.00–2.67)	(−3.0–1.0)
Block design	48	59	14
normative scaled score	5.83 ± 4.05	4.25 ± 3.21	3.43 ± 3.30
mean=	6	3	2.5
10 ± 15	(1–16)	(1–18)	(1–13)
Beery VMI	49	36	6
normative standard score	80.02 ± 20.86	72.72 ± 18.97	61.83 ± 19.96
mean =	80	74.5	55
100 ± 15	(45–123)	(45.0–124.0)	(45–88)

Table 3 (continued)

	ASD citrullinemia	ASA	ARGD
Motor domain	23	31	9
z-score	−1.83 ± 2.13	−2.60 ± 1.38	−3.90 ± 1.37
	−1.62	−2.42	−4.4
	(−6.67–1.05)	(−5.70–0.27)	(−5.5- -1.4)
Grooved pegboard (dominant hand) normative	22	26	8
standard score mean = 100 ± 15	69.72 ± 38.90	51.81 ± 24.10	32.25 ± 37.10
	75.5	46.5	24.5
	(0–125)	(20–109)	(0–117)
Grooved pegboard (non-dominant hand)	22	26	8
normative standard score mean = 100 ± 15	67.86 ± 36.73	55.54 ± 28.18	28.63 ± 37.04
	71	62	18
	(0–115)	(9–104)	(0–106)
Grip strength (dominant hand) normative	21	28	9
standard score mean =	80.38 ± 31.90	67.43 ± 27.55	49.44 ± 16.35
100 ± 15	88	71.5	51
	(0–121)	(11–111)	(24–72)
Grip strength (non-dominant hand) normative	22	28	9
standard score mean = 100 ± 15	78.96 ± 31.37	69.71 ± 27.01	49.56 ± 19.02
	86.5	74	57
	(0–119.0)	(3–102)	(10–71)
Memory domain	32	30	6
z-score	−0.63 ± 1.29	−1.61 ± 0.99	−1.07 ± 1.11
	−0.8	−1.34	−1.2
	(−2.9–3.6)	(−4.00–0.07)	(−2.4–0.2)
CVLT list A trial 1	20	22	1
z-score	0 ± 1.17	−1.02 ± 1.26	1.50
	0	−0.75	
	(−2.5–2.0)	(−3.50–1.00)	
CVLT list A trial 5	20	22	1
z-score	−0.40 ± 1.49	−2.09 ± 1.37	−0.5
	−0.5	−2	
	(−4.0–2.0)	(−5–0)	
BRIEF working memory	31	28	6
normative standard score	61.13 ± 12.20	64.38 ± 13.08	60.17 ± 9.56
mean = 50 ± 10	59	65	57
	(36–84)	(38–89)	(48–74)
REY Osterreith immediate recall normative standard score	14	10	1
mean = 50 ± 10	43.50 ± 22.88	34.8 ± 16.08	20
	35.5	28.5	
	(19.0–86.0)	(20.0–68.0)	

n = number of cases; IQ = intelligence quotient; ABAS GAC = Adaptive Behavior Assessment System, General Adaptive Composite; BRIEF GEC = Behavior Rating Inventory of Executive Function, Global Executive Composite; CBCL = Child Behavior Checklist; VMI=Visual Motor Integration; CVLT = California Verbal Learning test

Results

The total sample included 145 individuals 3 years of age and older, with a mean age of 13.78 years. As shown in Table 2, there were 63 males and 82 females, with the majority diagnosed with ASD ($n = 64$) or ASA (65) and 16 with ARG. In all three distal disorders, onset of symptoms occurred for the majority of babies after the newborn period, with the mean

age of urea cycle diagnosis occurring at just under 2 years of age. In this sample 13 (20%) with ASD and 11 (17%) individuals with ASA received liver transplant. Newborn screening, which was instituted during the period of expanded newborn screening (2004 in most states) led to early identification of 25% of infants with ASD and 29% of infants with ASA but none with ARGD. In general, demographic characteristics and health history as well as age at participation in the study were similar for the ASD and ASA groups, while the ARGD group included fewer cases, was older at time of symptom onset, diagnosis, and neuropsychological testing, did not come to attention through newborn screening, and did not undergo liver transplantation.

Results from the most recent neuropsychological evaluation are presented in Table 3.

Mean full scale IQ was in the range of intellectual disabilities (IQ < 71) for the ASA and ARGD groups and in the borderline range (IQ < 86) for the ASD group. The greatest deficits were noted in the visual performance and motor skills domains for all groups. Fig. 1 illustrates the distribution of full scale IQ for each disorder.

Laboratory values are presented in Table 4. All groups included individuals with hyperammonemic episodes. Mean ammonia levels were lower on the day of neuropsychological testing (for the few who received blood tests on that day) than lifetime mean levels, which were > 100 μmol/L for the ASD and ASA groups and were also relatively elevated for the ARG group. Standard deviation levels, as a measure of variability, were > 100 μmol/L for ASD and ASA groups. All groups included individuals who had "spikes" of ammonia > 10 μmol/L compared to their mean scores.

Lifetime mean laboratory values were inter-correlated, with citrulline most closely associated with ammonia. In ASD, lifetime mean citrulline levels correlated with lifetime mean ammonia ($r = 0.42$, $p = 0.0008$). In ASA, lifetime mean citrulline correlated with mean ammonia ($r = 0.33$, $p = 0.11$) and mean arginine ($r = 0.34$, $p = 0.007$). In ARGD, lifetime mean citrulline correlated with mean ammonia ($r = 0.42$, $p = 0.02$).

Table 5 presents the associations between biomarkers and neuropsychological domains with correlation coefficients above 0.30. Cumulative exposure emerged as one of the most sensitive indicators of neuropsychological outcomes. Concurrent blood levels of the biomarkers examined were not correlated with measures of functioning for any disorder in any domain.

For ASD, cumulative exposure to ammonia and citrulline were the most reliable indicators of poorer functioning in every domain, suggesting that long-term exposure poses the greatest risks. Glutamine cumulative exposure was related to global functioning but not to specific functional domains. The total number of hyperammonemic episodes correlated with visual performance and motor abilities, but not with the verbal composite score. Arginine, which tends to be close to normal in ASD, was not associated with performance in any of the neuropsychological domains.

For ASA, the most sensitive biochemical markers for global functioning and verbal abilities were ammonia, glutamine, and citrulline. All were in the expected direction, with lower laboratory values correlating with better scores on the neuropsychological tests. Scores in the visual performance and motor domains correlated with arginine levels, indicating that high cumulative arginine exposure and high variability were associated with poorer motor functioning scores. None of the biomarkers correlated with the composite memory score.

For ARGD, cumulative exposure to arginine, ammonia, and citrulline correlated at least at a $r = 0.30$ level with global

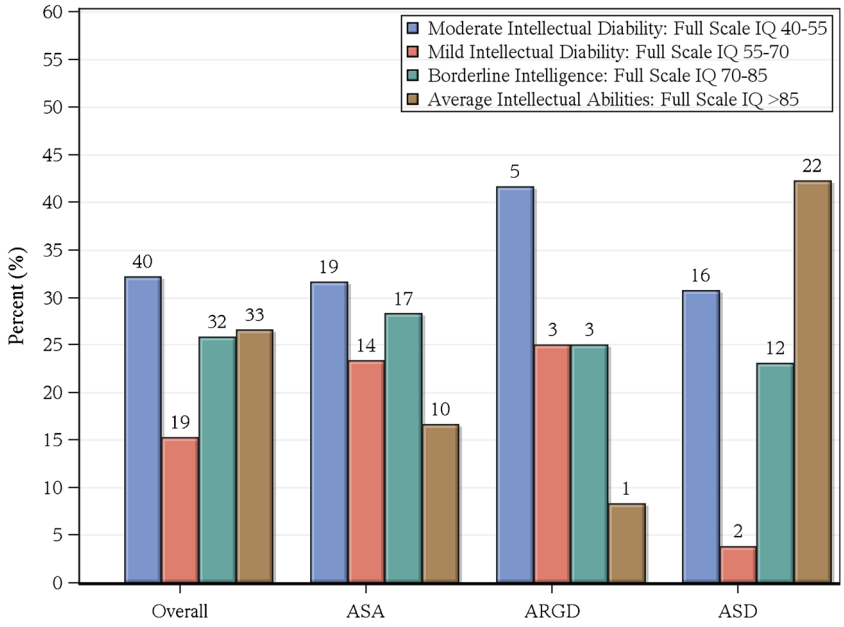

Fig. 1 Distribution of full scale IQ for overall sample and for argininosuccinic acidemia (ASA), arginine deficiency (ARGD), and argininosuccinic lyase deficiency (ASD). (Numbers above bar lines indicate sample size)

Table 4 Laboratory results for biomarkers (ammonia, glutamine, citrulline and arginine (n, mean ± standard deviation, median, (range))

Biomarker	ASD citrullinemia	ASA	ARGD
Ammonia (Reference range: 28–80 μmol/L)			
Number of hyperammonemic episodes	63	63	15
	5.6 ± 7.08	4.14 ± 6.09	3.93 ± 5.82
	3	1	1
	(1–32)	(1–27)	(1–22)
Concurrent level	23	20	2
	44.71 ± 43.49	25.08 ± 17.28	5.28, 67.0
	30	22.5	36.14
	(9–206)	(9.0–82.0)	(5.28–67.0)
Mean lifetime level	62	59	16
	127.22 ± 110.51	139.59 ± 164.89	78.87 ± 51.84
	108.86	80	77.5
	(6.30–539.25)	(1–882)	(5.28–169.33)
Standard deviation score*	53	48	12
	151.06 ± 168.03	169.02 ± 251.57	46.46 ± 34.87
	101.22	83.76	35.31
	(4.03–780.32)	(3.27–1164.06)	(5.80–108.93)
Number of "spikes"**	63	60	16
	4.56 ± 6.16	3.47 ± 5.67	2.38 ± 4.77
	2	1	0.5
	(0–30)	(0–25)	(0–19)
Glutamine reference range: 332–1084 μmol/L			
Concurrent	23	24	3
	603.55 ± 1.95.49	638.31 ± 198.80	515.00 ± 74.28
	606	626	488
	(121–932)	(36–1020)	(458–599)
Mean	63	63	16
	687.16 ± 205.71	706.94 ± 222.85	632.71 ± 167.81
	657.65	682.36	594.6
	(347.0–1442.0)	(334.0–1997.0)	(374.5–1026.2)
Standard deviation score	61	55	16
	197.78 ± 174.11	231.45 ± 347.49	161.62 ± 77.86
	163.54	139.63	146.54
	(1.41–1223.86)	(24.04–2444.84)	(61.94–326.75)
Citrulline reference range: 2–50 μmol/L			
Concurrent	23	24	3
	1804.48 ± 1284.18	159.68 ± 51.15	28.33 ± 5.03
	2161.0	156	29.0
	(86.4–4766.0)	(77–284)	(23–33)
Mean	62	63	16
	1857.97 ± 1141.31	194.37 ± 98.06	24.16 ± 5.43
	1836.56	168.68	23.44
	(71.31–4752.00)	(42.00–554.33)	(17.76–34.67)
Standard deviation score	61	54	16
	700.62 ± 810.50	92.36 ± 105.43	6.16 ± 4.07
	508.00	51.01	5.81
	(26.66–5106.23)	(6.01–566.31)	(0–15.82)

Table 4 (continued)

Biomarker	ASD citrullinemia	ASA	ARGD
Arginine reference range: 16–149 μmol/L			
Concurrent	23	24	3
	69.15 ± 50.90	76.39 ± 45.89	388.67 ± 85.19
	54.7	64	362
	(5.0–194.0)	(20–220)	(320–484)
Mean	62	63	16
	128.17 ± 185.30	107.60 ± 66.51	371.16 ± 121.26
	101.94	95.8	353.98
	(45.30–1507.33)	(26.6–444.0)	(181—535.33)
Standard deviation score	61	54	16
	96.29 ± 319.46	71.169 ± 69.61	105.86 ± 64.13
	44.04	55.46	113.38
	(2.92–2511.93)	(4.24—445.27)	(0–283.89)

n = number of participants

*Standard deviation score = the mean degree of dispersion in laboratory values around the mean value for the individual participant was labeled the standard deviation score

**Ammonia "spikes" = an ammonia level > 10 μmol/L above the mean for the individual participant

functioning. In other domains, exposure to elevated arginine and citrulline correlated with poorer functioning. Hyperammonemic episodes were associated with poorer scores on tests of verbal skills and visual performance, but paradoxically, they were associated with better performance on motor tasks.

Discussion

This study provides natural history data related to demographics, metabolic biomarkers, and neuropsychological outcomes in three distal urea cycle disorders. As expected, each disorder was characterized by unique biochemical profiles. With ASD, citrulline was significantly elevated while in ASA, ammonia-related biomarkers were most notable, and arginine was conspicuous in ARGD. The biomarkers investigated here are not independent variables. Although clinical guidelines were developed over 15 years ago (Summar and Tuchman 2001), treatment practices appear to vary considerably. Data from this study indicate that arginine and citrulline as well as ammonia and glutamine reflect a patient's risk for adverse neuropsychological effects and may indicate a need for treatment modifications. Similarly, neuropsychological functioning domains are not independent variables. However, when considered alone and in relation to biochemical parameters, they provide information that may be useful in the selection of clinical outcome variables.

For ASD, poor performance in every domain was associated with cumulative exposure to citrulline as well as to cumulative exposure to ammonia. This is in contrast to a recent publication reporting that neuropsychological function in ASD did not correlate with episodes of hyperammonemia (Baruteau et al 2017). This may be due to the fact that isolated episodes of hyperammonemia are not as deleterious as cumulative exposure to moderately high levels of ammonia or citrulline.

For ASA, ammonia clearly related to aspects of cognitive outcome, but so did glutamine. Others have suggested that elevated glutamine serves as a harbinger for sudden hyperammonemia (Lee et al 2016). Our study suggests that glutamine can also be used as a marker for neuropsychological functioning. This is in accordance with Gunz et al (2013) who reviewed studies using magnetic resonance imaging in children with neonatal onset UCDs. They identified abnormalities in the cerebral cortex, internal capsule, basal ganglia, thalami, and brain stem in association with higher glutamine levels and poorer neurological outcomes. In addition, and not reported previously, our study showed that elevated mean lifetime and cumulative citrulline levels were highly correlated with intellectual functioning, verbal skills, and visual performance. Furthermore, poor motor skills appeared to be affected by arginine, which was implicated when levels were low.

While each group experienced significant neuropsychological deficits, ARGD appeared to confer the greatest risk for low IQ and poor performance in every domain.

There was preliminary evidence that variability in each of the biomarkers led to greater risks for neuropsychological deficits. The impact of elevated ammonia, even when below the cut-off for hyperammonemia (< 100 μmol/L), should not be underestimated in this disorder. Moreover, elevations in arginine and the standard deviation of arginine levels tended to be associated with poorer functioning in the global and memory domains. On the other hand, the positive correlations between

Table 5 Associations between biomarkers and composite neuropsychological domain scores with correlation coefficients equal to or greater than +/− 0.30 for argininosuccinic acid synthetase deficiency (ASD) ($n = 60$), argininosuccinic acid lyase deficiency (ASA) ($n = 62$), and arginase deficiency (ARGD) ($n = 16$)

	ASD (citrullinemia)	
Global functioning ($n = 60$)	Correlation	*p*-value
# hyperammonemic episodes	−0.32	0.011
Ammonia mean	−0.40	0.002
Ammonia cumulative exposure*	−0.65	<0.0001
# ammonia "spikes"	−0.33	0.011
Glutamine cumulative exposure	−0.34	0.008
Citrulline cumulative exposure	−038	0.003
Verbal composite ($n = 58$)		
Ammonia mean	−0.59	<0.0001
Ammonia cumulative exposure*	−0.55	<0.0001
Ammonia standard deviation	−0.46	0.0011
Citrulline cumulative exposure	−0.35	0.008
Memory composite ($n = 31$)		
None of the biomarkers met criteria		
Visual performance ($n = 47$)		
# hyperammonemic episodes	−0.43	0.002
Ammonia mean	−0.61	<0.0001
Ammonia cumulative exposure*	−0.62	<0.0001
Ammonia standard deviation	−0.44	0.006
# ammonia "spikes"	−0.50	0.0003
Citrulline mean exposure	−0.40	0.005
Citrulline cumulative exposure	−0.43	0.002
Motor composite ($n = 23$)		
# hyperammonemic episodes	−0.59	0.004
Ammonia cumulative exposure*	−0.68	0.0004
# ammonia "spikes"	−0.59	0.003
Citrulline mean	−0.62	0.002
Citrulline cumulative exposure	−0.61	0.002
	ASA	
Global functioning ($n = 62$)	Correlation (r)	p-value
# hyperammonemic episodes	−0.35	0.005
Ammonia mean	−0.35	0.007
Ammonia cumulative exposure*	−0.42	0.003
Glutamine cumulative exposure	−0.40	0.001
Citrulline mean	−0.44	0.0003
Citrulline cumulative exposure	−0.48	<0.0001
Verbal composite ($n = 62$)		
# hyperammonemic episodes (n = 62)	−0.40	0.001
Ammonia mean	−0.44	0.0006
Ammonia cumulative exposure*	−0.38	0.006
Number of ammonia "spikes"	−0.39	0.002
Glutamine cumulative exposure	−0.35	0.005
Citrulline mean	−0.53	<0.0001
Citrulline cumulative exposure	−0.41	0.001
Memory composite ($n = 28$)		
None of the biomarkers met criteria		
Visual performance ($n = 57$)		
Ammonia mean	−0.37	0.007
Ammonia cumulative exposure*	−0.35	0.0120
Citrulline mean exposure	−0.42	0.001
Citrulline cumulative exposure	−0.36	0.007
Motor composite ($n = 30$)		
Arginine cumulative exposure	−0.47	0.009
Arginine standard deviation	−0.49	0.011
	ARGD	
Global functioning ($n = 16$)	Correlation (r))	p-value
Ammonia cumulative exposure*	−0.39	0.13
Citrulline cumulative exposure	−0.32	0.23
Arginine cumulative exposure	−0.36	0.17
Verbal composite ($n = 16$)		
# hyperammonemic episodes	−0.38	0.17
Memory composite ($n = 6$)		
Glutamine mean	−0.37	0.47
Citrulline mean	−0.58	0.23
Arginine mean	−0.41	0.43
Arginine standard deviation	−0.63	0.26
Visual performance ($n = 14$)		
# hyperammonemic episodes	−0.38	0.20
Motor composite ($n = 9$)		
Ammonia mean	0.38	0.35
Ammonia standard deviation	0.30	0.56
# ammonia "spikes"	0.61	0.11
Citrulline mean	−0.30	0.43
Citrulline standard deviation	−0.47	0.20

*Cumulative exposure was defined as the mean exposure X Age up until and including the age at testing. In liver transplant cases, ammonia cumulative exposure was defined as the mean exposure X age up until the time of liver transplant

the standard deviation scores for citrulline, glutamine, and ammonia on the verbal and memory domains and elevated ammonia on motor functioning are difficult to explain except by recognizing the possibility of spurious correlations derived from the small sample sizes.

This study is limited by small sample sizes as well as heterogeneity in severity of disease (as illustrated by variability in number of hyperammonemic episodes and age at diagnosis). Another shortcoming is the lack of consideration of treatment variables and other potential covariates, such as branched chain amino acids. Small sample sizes also led to a decision to report only correlations of 0.30 or greater. Methods that would have reduced the number of analyses by examining ratios for biochemical parameters or measures of global functioning were considered

but would not have allowed for identification of individual biomarkers or specific neuropsychological domains as targets for evaluating clinical outcomes. Since the elapsed time following a hyperammonemic episode was not controlled for, a lack of correlation between concurrent ammonia levels or other biomarkers and scores on cognitive tests needs further investigation. Similarly, an alternative cut-off definition for the number of ammonia "spikes" might have been more informative and requires further study.

Despite the limitations inherent in natural history studies such as this one, (Shapiro et al 2016), the following general findings from our study may be usefully taken into account when treating these urea cycle disorders and when selecting endpoints in clinical trials.

1. Metabolic biomarkers obtained at the time of neuropsychological testing do not correlate with performance.
2. Cumulative exposure to potentially toxic metabolites is closely associated with performance on cognitive and other neuropsychological tests. This may explain why early development in ASA and ARGD appears to be closer to that of typically developing children (Waisbren et al 2016).
3. While ammonia levels remain prominent as prognostic biomarkers, other biomarkers may be equally valuable as correlates of neuropsychological functioning. Thus, the study confirms the need for well-controlled management of all biochemical abnormalities associated with the disorder and suggests that the broad biochemical profile should be assessed in clinical trials.
4. Verbal and visual performance composite scores may be important functional outcome measures. Individuals with ASA, ASD, and ARG experienced greatest deficits in the motor skills domain. However, the biomarkers in this study were not as strongly associated with composite motor scores as with the verbal and visual performance composite scores. Memory, although also a particular deficit, was not strongly associated with biomarkers. The likely explanation for this is that early onset hyperammonemia causes irreversible damage to the brain in association with motor and memory functions, as was suggested by the extensive analysis of cases followed by the European IMD Consortium (Posset et al 2016).
5. The relevance of the standard deviation score and ammonia spikes in ASD and ASL underscores the need for stability in metabolic control.

The findings of our study empirically substantiate the common sense conclusions that patients with any metabolic disorder do better when diagnosed sooner and remain under good metabolic control. Novel treatments for these urea cycle disorders are now being investigated at an impressive rate. The need to bring these treatments to market could not be greater. This study provides insights into potential targets for therapies and suggests potential neuropsychological endpoints to determine efficacy in clinical trials for distal UCDs.

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Compliance with ethical standards

Conflict of interest Susan Waisbren consults for Dimension Therapeutics.

Dr. Cederbaum consults for Aeglea Biotherapeutics.

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