Published in final edited form as:

J Pediatr. 2013 February ; 162(2): 324–9.e1. doi:10.1016/j.jpeds.2012.06.065.

Clinical outcomes of neonatal onset proximal versus distal urea cycle disorders do not differ

Nicholas Ah Mew, MD¹, Lauren Krivitzky, PhD², Robert McCarter, ScD¹, Mark Batshaw, MD¹, and Mendel Tuchman, MD^{1,*} on behalf of the Urea Cycle Disorders Consortium of the Rare Diseases Clinical Research Network

¹Center for Genetic Medicine and Center for Clinical and Community Research, Children's National Medical Center, Department of Pediatrics, The George Washington University, Washington, DC 20010, USA

²Neuropsychology & Assessment, Department of Child and Adolescent Psychiatry and Behavioral Sciences, The Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA

Abstract

Objective—To compare the clinical course and outcome of patients diagnosed with one of four neonatal onset urea cycle disorders (UCDs), carbamyl phosphate synthase 1 (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthase (AS) or argininosuccinate lyase (AL) deficiency.

Study design—Clinical, biochemical, and neuropsychological data from 103 subjects with neonatal onset UCDs were derived from the Longitudinal Study of Urea Cycle Disorders, an observational protocol of the Urea Cycle Disorder Consortium, one of the Rare Disease Clinical Research Networks.

Results—88% of subjects presented clinically by age 7 days. Peak ammonia level was 963 μ M in patients with proximal UCDs (CPSD or OTCD), compared with 589 μ M in ASD and 573 μ M in ALD. 25% of subjects with CPSD/OTCD, 18% with ASD, and 67% with ALD had a honeymoon period, defined as time interval from discharge from initial admission to subsequent admission for hyperammonemia, greater than 1 year. The proportion of patients with a poor outcome (IQ/DQ < 70) was greatest in ALD (68%), followed by ASD (54%) and CPSD/OTCD (47%). This trend was not significant, but was observed in both the under age 4 years and 4 years and older category. Poor cognitive outcome did not correlate with peak ammonia level or length of initial admission.

© 2012 Mosby, Inc. All rights reserved

Corresponding author: Nicholas Ah Mew, MD Children's National Medical Center 111 Michigan Ave, NW Washington, DC Phone: (202) 476-5863 Fax: (202) 476-2390 naahmew@cnmc.org.

*List of members of the Urea Cycle Disorders Consortium of the Rare Diseases Clinical Research Network is available at www.jpeds.com (Appendix).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors declare no conflicts of interest.

Conclusions—Neurocognitive outcomes do not differ between those with proximal and distal UCDs. Factors other than hyperammonemia may contribute to poor neurocognitive outcome in the distal urea cycle disorders.

Keywords

carbamyl phosphate synthetase I; ornithine transcarbamylase; argininosuccinic acid lyase; argininosuccinic acid synthetase; hyperammonemia; inborn errors of metabolism; rare disease; clinical research

The urea cycle consists of five biochemical steps required for the conversion of ammonia to urea[1]. A deficiency of any of these enzymes reduces or halts flux through the urea cycle, and typically results in acute hyperammonemia. Complete deficiencies of the proximal urea cycle enzymes carbamyl phosphate synthetase I (CPS1; EC 6.3.4.16) and ornithine transcarbamylase (OTC; EC 2.1.3.3) (CPSD or OTCD) are considered to cause more severe hyperammonemia than the distal urea cycle enzymes such as AS or AL (ASD or ALD)[2]. In the latter disorders, the excretion of amino acids citrulline or ASA provides an alternative pathway for waste nitrogen excretion [2]. Intuitively, lower levels of blood ammonia should result in reduced neurotoxicity. However, there is some evidence that patients with ASD or ALD are equally or more severely impaired than those with CPSD or OTCD[3, 4] suggesting that there may be additional mechanisms of brain injury[5, 6].

We wished to determine whether infants with proximal UCD defects who present with neonatal disease experience different outcomes than did those with distal UCD defects. We utilized data collected as part of the Longitudinal Study of Urea Cycle Disorders[7, 8] [ClinicalTrials.gov ID: NCT00237315], a “natural history” observational protocol of the Rare Diseases Clinical Research Network's (RDCRN) Urea Cycle Disorders Consortium (UCDC), which consists of 14 research sites in academic centers within the United States, Canada, and Europe.

Herein, we summarize the clinical course and outcomes of patients with neonatal onset of CPS1, OTC, AS and AL deficiencies who survived beyond the neonatal period. We show that: (1) the majority of these patients present in the first week of life; (2) most of them have an event free “honeymoon” period of variable duration following the initial neonatal insult; and (3) neurocognitive outcomes do not differ between those with proximal and distal urea cycle deficiencies.

Methods

This report is based on a subset of participants enrolled in the previously described, IRB-approved UCDC longitudinal study[8, 9], which includes consented volunteers of all ages with a confirmed diagnosis of one the urea cycle enzyme defects. Only those diagnosed with CPSD, OTCD, ASD, and ALD contributed data to this report. As in the parent Longitudinal Study, data are derived from abstracts of medical records. At each encounter, the participants and/or guardian(s) were interviewed, the participant underwent a clinical evaluation, and a standard battery of laboratory investigations was performed. All study data

were entered into the UCDC database maintained by the Data Management and Coordinating Center (DMCC) of the RDCRN.

Participants underwent neuropsychological and developmental evaluation at the time of enrollment, and then at set ages throughout the study period (6 months of age, 18 months, 4 years, 8 years, 15 years, and 18 years). The test captured neuropsychological and developmental function and was designed to be pertinent to the respective subject's age and developmental level for global intellectual/developmental functioning: Ages 0 to 3 years - Cognitive Scale from the Bayley Scales of Infant Development-Third Edition; Ages 3–5 - Full scale IQ (FSIQ) from the Wechsler Preschool and Primary Scales of Intelligence-Third Edition (WPPSI-III); Ages 6 to Adulthood - FSIQ from the Wechsler Abbreviated Scales of Intelligence (WASI). If an individual was unable to complete the appropriate test for his/her age group, a developmental quotient (DQ) score was obtained by dividing the age equivalence score on the Bayley Scales by the chronological age.

We obtained from the DMCC data for all enrolled participants with CPSD, OTCD, ASD, and ALD who were hospitalized in the first 30 days of life with either a peak recorded plasma ammonia level of $> 200 \mu\text{mol/l}$ (normal <35) or genetic analysis demonstrating mutations that had been previously reported to be associated with neonatal onset disease. Patients presenting after the first 30 days of life were excluded, as such a later presentation was suggestive of the presence of residual urea cycle function. Lastly, patients for whom data from the initial hospitalization were not available were also excluded. We also report on the current number of deceased patients known to have had neonatal-type disease who were not enrolled in the longitudinal study, but who we captured by review of medical records.

In the comparison of ammonia levels, we used chi square (for ordinal categories) and analysis of variance/covariance to compare maximal ammonia levels during the first hospitalization among the three diagnostic groups (CPSD and OTCD vs. ASD vs. ALD) to evaluate whether the proximal lesions (CPSD and OTCD) presented with higher ammonia levels than either of the distal lesions (ASD or ALD). When evaluating differences in interval length between first and second hospitalization for hyperammonemia, we used a competing outcome proportional hazard model to analyze whether this parameter differed by diagnostic group to account for groupwise differences in the presence of liver transplant as well as other covariables. When we compared neurocognitive development (DQ/IQ categories), we used logistic and ordinal logistic regression to compare the odds of greater DQ or IQ by diagnostic group. These analyses also controlled for other differences between groups.

Given the various psychometric tests used by the UCDC Longitudinal Study to assess neurocognitive function, we felt it was not appropriate to compare subject scores as a single continuous variable. Thus, we first created categories of cognitive/developmental functioning for the entire cohort (Poor vs. Non-Poor outcome) (Table I). A broader categorical score was then applied to individuals aged four years and older (Figure 1). Children under four years of age were not included in this second analysis as the concept of "IQ" in this age group is not easily applicable: Not only are scores at this younger age less stable and not highly predictive of later IQ, but the lowest attainable score on the Bayley

Scales is 55, and this “floor” reduces the comparison accuracy of individuals at the lower end of the spectrum. The categories for this second analysis were the same as those of a previously described model[10]

Results

Among the 500 participants enrolled in the Longitudinal Study, there were a total of 103 subjects with neonatal onset who met our inclusion criteria: 8 with CPSD, 30 with OTCD, 37 with ASD, and 28 with ALD. We combined CPSD and OTCD into a single “proximal UCD” entity of 38 subjects because these disorders are indistinguishable in terms of clinical presentation and amino acid abnormalities. Selected socio-demographic and clinical characteristics of the study sample grouped by diagnostic classification is provided in Table II (available at www.jpeds.com).

Parameters from the initial hospitalization are shown in Table III. The vast majority of subjects (91 of 103, 88%) presented clinically by 7 days of age. However, patients with proximal disorders presented earlier, had a higher peak ammonia level, and had a longer average length of stay. 11 patients (1 OTCD, 4 ASD, 6 ALD) were reportedly diagnosed by newborn screening. In AL deficiency, the initial serum transaminase levels were substantially higher than in the other groups ($p<0.001$ for ALT, $p=0.005$ for AST), consistent with prior reports[8].

Twenty-five patients (66%) with proximal urea cycle disorders underwent liver transplantation, compared with 10 patients (27%) with ASD and 10 (36%) with ALD ($p<0.001$) (Table IV; available at www.jpeds.com).

DNA testing is not part of the standard battery of investigations performed by the Longitudinal Study, but these data are captured by the study if clinically available. Mutation analysis was performed in 25 of 38 subjects (66%) with proximal UCDs, as compared with 9 of 37 with ASD (24%) and only 3 of 28 (11%) with ALD. These mutations were compared with published mutation updates[11–16] and the HGMD[17] (www.hgmd.org) and LOVD[18] (www.lovd.nl/2.0) mutation databases of the respective genes in an attempt to derive genotype-phenotype correlation data. Twenty-four patients (73% of those tested) had mutations that would presumably result in absent enzyme activity, such as nonsense or frameshift mutations, or those that were reported to be associated with “severe” or “neonatal” presentation[11–15]. Seven patients (all with OTCD) had mutations associated with a “late-onset” milder presentation[12, 14]. In 6 patients, the mutations were previously unreported, and a determination of severity could not be made on the basis of the genotype.

Several authors have previously noted, in patients with urea cycle disorders, a lengthy interval between the initial and subsequent episode of hyperammonemia, dubbed the “honeymoon period”[19, 20]. A Cox Proportional Regression survival plot by length of honeymoon period is defined as the time from discharge from the initial hospitalization until the second admission (Figure 2). The majority of patients with a proximal urea cycle disorder (75%) or ASD (82%) had a subsequent clinical hyperammonemia episode within one year of their initial hospitalization, compared with only 33% of patients with ALD.

However, in all three groups, there was a high risk of a subsequent hyperammonemia within the first 25 days following discharge from the initial hospitalization, followed by a near-linear rate of readmission. There were 6 patients with CPSD/OTCD who averted a second hyperammonemia event as a result of liver transplantation that occurred in the first year of life. In contrast, in only a single patient with ALD, and none with ASD, was liver transplantation performed sufficiently early to avert a second hyperammonemia episode.

We also examined the overall rate of hyperammonemia events in each cohort, through the date last seen, or if applicable, the date of liver transplantation. The frequency of events in the proximal UCDs and in ASD was compared with that of the ALD group, the cohort with the fewest events. In patients with proximal disorders, the risk of a hyperammonemia event was 2.2 times that observed in patients with ALD [95% CI: 1.2 – 4.0, $p=0.009$]. However, in patients with ASD, this risk was virtually identical (only 1.1 times) to that observed in ALD [95% CI: 0.62–2.0, $p=0.71$].

There were only 4 recorded deaths (4%) in this cohort of 103 patients. Two subjects, one with CPSD and one with ASD, died at 8 and 9 months of age respectively, following orthotopic liver transplantation. The risk of mortality following transplantation in our cohort was thus 2/45 (4.4%). Another subject was diagnosed with a 0.88 megabase contiguous deletion encompassing the OTC gene as well as other genes and thus co-morbidity may have played a role in his death at age 15 months. Finally, one female patient with ALD died at 21 years of age due to respiratory and liver failure. However, retrospective review at participating UCDC member institutions revealed an additional 9 deaths of UCD patients not enrolled in the Longitudinal Study: 2 CPSD, 5 OTCD, 1 ASD and 1 ALD. Death resulted from hyperammonemia in the first 45 days of life in all but two patients: one patient with OTCD who died of hyperammonemia at age 2 years, and one patient with ASD who died of complications from liver transplantation at 4 years of age. We analyzed neurocognitive testing results from 83 of 103 subjects in this cohort, including 30 with CPSD or OTCD, 28 with ASD, and 25 with ALD. Fifty-three of the 83 subjects were age 4 years or older when last tested. Twenty patients did not have testing due to death before testing was done (<6 months of age), refusal to participate or inability to schedule testing.

In children under the age of 4 years, the mean scores on the Bayley Cognitive Scale were consistently in the borderline range (ie, DQ 70–85) in the proximal and distal disorders (Table I), and they were not statistically different between the 3 diagnostic groups in this small sample. However, when comparing poor versus non-poor outcomes (Bayley Cognitive Score < 70 vs. 70), the ALD group surprisingly had the highest prevalence of poor outcome (50%) followed by the CPSD and OTC group (43%), and the ASD group (25%).

In subjects age 4 years and older, there were higher rates of poor outcomes (i.e., mild to severe intellectual disability) than in the younger group. This rate was 66% overall, and was again highest in the ALD group (76%), followed by ASD (70%), and CPSD/OTCD (50%) (Table I). However, a greater proportion of severe/profound intellectual disability was observed in the proximal disorders (44%) vs. ASD (25%) or ALD (18%) (Figure 1). When we adjusted the results for liver transplantation, peak ammonia at diagnosis, age at second

hospitalization, and frequency of hyperammonemia events, there was little evidence of an IQ difference between groups.

Fourteen subjects (17%) across all diagnoses had IQ scores in the average range (IQ > 90). Retrospective review could not identify factors relating to their initial admission that may have contributed to their better outcome. Median peak plasma ammonia level at admission was 583 $\mu\text{mol/l}$ in this group, which paradoxically included a subject with the highest peak ammonia level among all cases in this cohort (2684 $\mu\text{mol/l}$). Median length of stay during the first admission was 14 days.

Only 6 of the 11 subjects (3 ASD and 3 ALD) who were identified by newborn screening had neurocognitive testing. Among these, only 1 had a poor outcome, and the other 5 tested in either the low average or broadly average range. Median age at admission was 7 days.

Discussion

The ability to eliminate nitrogen waste (ammonia) is critical; therefore, in its absence, hyperammonemia almost always develops shortly after birth [1]. Though any defect in the urea cycle can cause hyperammonemia, we show that complete deficiencies of proximal urea cycle enzymes produce the highest levels of ammonia, a result which is consistent with earlier publications[2] because virtually no nitrogen is incorporated into, and excreted as, either urea or nitrogen-containing amino acids. We observed that lack of diagnostic amino acid markers makes DNA analysis the diagnostic method of choice in the proximal (66%) vs. distal (18%) UCDs.

A UCD phenotype described as “neonatal” has been used to identify the most severely affected children with UCDs - those thought to have a complete absence of one of the urea cycle enzymes. Residual urea cycle enzyme activity is believed to result in a “milder” clinical phenotype referred to as “late-onset” presentation. Here, we selected the widest recognized definition of neonatal (i.e., less than age 31 days). Yet, our analysis revealed that 39 of 103 (37%) affected neonates had developed hyperammonemia within the first 2 days of life and 91 (88%) had by 7 days (Table III). This observation is consistent with a previous report on OTCD in which virtually all hemizygous males with neonatal type presented by 5 days of age[21]. In our study, only 12% of subjects (12 of 103) first presented between 8 and 30 days of life. Among these, the 6 patients with proximal UCDs all had mutations in the OTC gene: 1 was a manifesting female heterozygote, 4 were hemizygous males with mutations previously identified as “late-onset” (ie, having residual enzymatic activity[12, 14]), and 1 was hemizygous for a previously undescribed OTC variant whose clinical presentation was consistent with a mild disorder (e.g. plasma ammonia level never exceeded 250 μM). Of the 6 patients with distal UCDs presenting after 1 week of age, 3 were identified by newborn screening, and their initial peak ammonia level was near our cut-off of 200 μM . Thus, our analysis strongly suggests that the most severe category of patients with UCDs - those patients with absent urea cycle function – present overwhelmingly within the first week of life. This very early presentation in complete defects coincides with the catabolic state and weight loss which persists in the few days postpartum even before large amount of milk intake could be established.

There were only 4 deaths recorded among the 103 patients discussed in this report. Even with expert care at a tertiary pediatric center, complete urea-cycle deficiencies would be expected to carry a higher mortality rate. In fact, we found 9 additional postmortem neonatal UCD cases, 7 of which occurred in the first 45 days of life. These numbers are likely incomplete, and there may be other early mortalities from UCD that have been overlooked. The likelihood of a sampling bias is supported by the low number of severe neonatal OTCD cases, which only accounted for 30 of 200 symptomatic OTCD participants in the longitudinal study. This low proportion is in stark contrast with the much higher frequency of neonatal OTCD cases reported by our reference laboratory [8].

Several authors have previously noted a lengthy interval between the neonatal, and subsequent episode of hyperammonemia, dubbed the “honeymoon period”[19, 20]. This period of clinical stability, even in those infants with a severe disorder, is posited to be as a result of the rapid growth and increased nitrogen requirements of young children. Our analysis demonstrates that this phenomenon may not broadly apply to all infants with UCDs. Among ASD and the proximal disorders, there was a nearly 20% recurrence risk in the first month following discharge but after the first month, this risk seems markedly reduced. In addition, the risk of a second hyperammonemia episode in the first year of life is lower in ALD as compared with ASD or proximal disorders.

These data would also suggest that if performed early, liver transplantation, could prevent a second hyperammonemic episode. Indeed, 4 of 5 proximal UCD subjects who avoided any hyperammonemia episodes after the newborn period had a hepatic transplant by 101 days-of-life.

As previously reported, the likelihood of intellectual deficits is quite high in individuals with neonatal onset UCDs[3, 10, 22, 23]. In our cohort, 55% demonstrated mild to profound cognitive deficits but we found little difference in cognitive outcome between proximal and distal UCD diagnoses. We show that by virtually any benchmark (age at presentation, peak ammonia level, length of “honeymoon period”, number of hyperammonemia episodes) the proximal urea cycle disorders have a more severe clinical course than the distal UCD. One might thus intuit that neurodevelopmental outcome should be worse in the proximal disorders. However, this hypothesis was not borne out by our study. In fact, although not statistically significant, patients with ALD had the greatest risk of poor outcome (68%). This supports the notion that there may be metabolic toxins other than ammonia, such as argininosuccinate, which play a role in the neurodevelopmental outcome in this condition.

It is surprising that poor cognitive outcome did not correlate with peak ammonia level or length of initial hospitalization. It may be that once plasma ammonia level is increased beyond a certain threshold, further elevation has no clinical implications. Alternatively, as previously reported[3], it might instead be that the number of days or episodes above this threshold is of greater significance.

It is encouraging to note that only 1 of 6 patients identified by newborn screening with available neurocognitive data had a poor cognitive outcome. There could be two interpretations of this finding. It might represent a bias of selection which is present in any

preclinical mass screening because those who are more severely affected might present prior to the reporting of newborn screening results. This is supported by the later median age of presentation in these patients versus the entire study sample (7 days vs. 2 days). Alternatively, early therapy and avoidance of episodes may improve neurocognitive outcome.

In sum, this report describes a large cohort of patients with neonatal UCDs. Although still in its early stages, our approach to multicenter collaborative clinical research has already provided insights into the natural history of these ultra-rare conditions. This is also a first attempt to analyze factors affecting neurocognitive outcome among patients with different UCDs. Continued enrollment and study will improve understanding of the neuropsychological strengths and weaknesses in the UCDs, will help elucidate which factors improve neurocognitive outcome, and should result in enhancements in the standard of care for these conditions.

Acknowledgments

We thank Dr Mary Lou Oster-Granite and Dr Melissa Parisi, at the National Institute of Child Health and Human Development for their scientific advice and support. We also acknowledge Jianping He, at Children's National Medical Center, for his contribution to statistical analyses, Jennifer Seminara, at Children's National Medical Center, for her assistance with data acquisition and validation, and Dr. Hye-Seung Lee, at the Data Management and Coordinating Center at the University of South Florida, for her biostatistics and informatics expertise.

Supported been provided by National Institute of Child Health and Human Development (U54HD061221), National Center for Advancing Translational Sciences (UL1RR031988 and UL1TR000075), National Institutes of Health, O'Malley Family Foundation, and the Kettering Fund. The contents of this report are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Abbreviations

ABAS-II	Adaptive behavior assessment system-second edition
AL(D)	Argininosuccinate lyase (deficiency)
AS(D)	Argininosuccinate synthetase (deficiency)
ASA	Argininosuccinate
CPS1	Carbamyl phosphate synthetase I
CPSD	Carbamyl phosphate synthetase I deficiency
DMCC	Data Management and Coordinating Center
DQ	Developmental quotient
FSIQ	Full scale intelligence quotient
IQ	Intelligence quotient
OTC(D)	Ornithine transcarbamylase (deficiency)
PIQ	Performance intelligence quotient
NAGS	N-acetylglutamate synthase
NIH	National Institutes of Health

RDCRN	Rare Disease Clinical Research Network
UCD	Urea cycle disorders
UCDC	Urea Cycle Disorders Consortium
VIQ	Verbal intelligence quotient
WPPSI	Wechsler preschool and primary scale of intelligence
WASI	Wechsler abbreviated scale of intelligence

References

- [1]. Summar M, Tuchman M. Proceedings of a consensus conference for the management of patients with urea cycle disorders. *J Pediatr.* 2001; 138:S6–10. [PubMed: 11148544]
- [2]. Batshaw ML. Hyperammonemia. *Curr Probl Pediatr.* 1984; 14:1–69. [PubMed: 6510017]
- [3]. Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED. Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. *N Engl J Med.* 1984; 310:1500–5. [PubMed: 6717540]
- [4]. Maestri NE, Hauser ER, Bartholomew D, Brusilow SW. Prospective treatment of urea cycle disorders. *J Pediatr.* 1991; 119:923–8. [PubMed: 1720458]
- [5]. Nagamani SC, Erez A, Lee B. Argininosuccinate lyase deficiency. *Genet Med.* 2012; 14:501–7. [PubMed: 22241104]
- [6]. Cederbaum SD, Shaw KN, Valente M, Cotton ME. Argininosuccinic aciduria. *Am J Ment Defic.* 1973; 77:395–404. [PubMed: 4706398]
- [7]. Seminara J, Tuchman M, Krivitzy L, Krischer J, Lee HS, Lemons C, et al. Establishing a consortium for the study of rare diseases: The Urea Cycle Disorders Consortium. *Mol Genet Metab.* 2010; 100(Suppl 1):S97–105. [PubMed: 20188616]
- [8]. Tuchman M, Lee B, Lichter-Konecki U, Summar ML, Yudkoff M, Cederbaum SD, et al. Cross-sectional multicenter study of patients with urea cycle disorders in the United States. *Mol Genet Metab.* 2008; 94:397–402. [PubMed: 18562231]
- [9]. Seminara J, Tuchman M, Krivitzy L, Krischer J, Lee HS, Lemons C, et al. Establishing a consortium for the study of rare diseases: The Urea Cycle Disorders Consortium. *Mol Genet Metab.* 100(Suppl 1):S97–105. [PubMed: 20188616]
- [10]. Krivitzy L, Babikian T, Lee HS, Thomas NH, Burk-Paull KL, Batshaw ML. Intellectual, adaptive, and behavioral functioning in children with urea cycle disorders. *Pediatr Res.* 2009; 66:96–101. [PubMed: 19287347]
- [11]. Linnebank M, Tschiedel E, Haberle J, Linnebank A, Willenbring H, Kleijer WJ, et al. Argininosuccinate lyase (ASL) deficiency: mutation analysis in 27 patients and a completed structure of the human ASL gene. *Hum Genet.* 2002; 111:350–9. [PubMed: 12384776]
- [12]. Tuchman M, Jaleel N, Morizono H, Sheehy L, Lynch MG. Mutations and polymorphisms in the human ornithine transcarbamylase gene. *Hum Mutat.* 2002; 19:93–107. [PubMed: 11793468]
- [13]. Trevisson E, Salviati L, Baldoin MC, Toldo I, Casarin A, Sacconi S, et al. Argininosuccinate lyase deficiency: mutational spectrum in Italian patients and identification of a novel ASL pseudogene. *Hum Mutat.* 2007; 28:694–702. [PubMed: 17326097]
- [14]. Yamaguchi S, Brailey LL, Morizono H, Bale AE, Tuchman M. Mutations and polymorphisms in the human ornithine transcarbamylase (OTC) gene. *Hum Mutat.* 2006; 27:626–32. [PubMed: 16786505]
- [15]. Engel K, Hohne W, Haberle J. Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. *Hum Mutat.* 2009; 30:300–7. [PubMed: 19006241]
- [16]. Haberle J, Shchelochkov OA, Wang J, Katsonis P, Hall L, Reiss S, et al. Molecular defects in human carbamoyl phosphate synthetase I: mutational spectrum, diagnostic and protein structure considerations. *Hum Mutat.* 2011; 32:579–89. [PubMed: 21120950]

- [17]. Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, et al. The Human Gene Mutation Database: 2008 update. *Genome Med.* 2009; 1:13. [PubMed: 19348700]
- [18]. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. *Hum Mutat.* 2011; 32:557–63. [PubMed: 21520333]
- [19]. Leonard JV. The nutritional management of urea cycle disorders. *J Pediatr.* 2001; 138:S40–4. discussion S4–5. [PubMed: 11148548]
- [20]. Lee B, Singh RH, Rhead WJ, Sniderman King L, Smith W, Summar ML. Considerations in the difficult-to-manage urea cycle disorder patient. *Crit Care Clin.* 2005; 21:S19–25. [PubMed: 16227112]
- [21]. McCullough BA, Yudkoff M, Batshaw ML, Wilson JM, Raper SE, Tuchman M. Genotype spectrum of ornithine transcarbamylase deficiency: correlation with the clinical and biochemical phenotype. *Am J Med Genet.* 2000; 93:313–9. [PubMed: 10946359]
- [22]. Gropman AL, Batshaw ML. Cognitive outcome in urea cycle disorders. *Mol Genet Metab.* 2004; 81(Suppl 1):S58–62. [PubMed: 15050975]
- [23]. Bachmann C. Outcome and survival of 88 patients with urea cycle disorders: a retrospective evaluation. *Eur J Pediatr.* 2003; 162:410–6. [PubMed: 12684900]

Figure 1. Neurodevelopmental outcome of subjects age 4 years or older by diagnosis

Each diagnostic group - proximal UCD (CPSD/OTCD), ASD, and ALD - as well as the total neonatal UCD cohort, is stratified by neurodevelopmental outcome, characterized as follows:

- **Profound/Severe Range of Disability:** Subject was not testable by traditional IQ testing for their age range (i.e., WPPSI for 3–5 year olds and WASI for 6 years and older). Bayley Scales were instead administered to derive a developmental quotient (DQ).
- **Mild-Moderate Range of Disability:** FSIQ Score of 45–69. If no FSIQ was available, Verbal Intelligence Quotient (VIQ) or Performance Intelligence Quotient (PIQ) was instead used to determine categorization.
- **Low Average/Borderline Functioning:** FSIQ Score of 70–89. If no FSIQ was available, VIQ or PIQ was instead used to determine categorization.
- **Broadly Average:** FSIQ Score of 90–109. If no FSIQ is available, VIQ or PIQ was instead used to determine categorization.
- **Above Average:** FSIQ ≥ 110. No subject met this criterion.

Figure 2. Length of the Honeymoon Period

Cox Proportional Regression of “Honeymoon Period”, defined as time interval from discharge from initial hospitalization to subsequent admission for hyperammonemia, controlling for peak ammonia at first admission, years since availability of ammonia scavengers (July 1996), paternal education, and family income. Liver transplant was considered as a competing risk. The proportion of patients with no second admission over length of honeymoon period is illustrated for each diagnostic category - CPSD/OTCD, ASD, and ALD.

Table 1

Intellectual/developmental outcome by diagnosis

	CPSPD/OTCD (n=38)	ASD (n=37)	ALD (n=28)	All Subjects (n=103)
N with neuropsychological testing	30	28	25	83
% with poor cognitive outcomes*	47%	54%	68%	55%
N under age 4 years	14	8	8	30
Mean age (years)	1.6	1.6	1.8	1.6
Mean Bayley cognitive scores	76.4	79.6	70.6	75.8
% with poor cognitive outcomes	43%	25%	50%	40%
N ages 4 years and above	16	20	17	53
Mean age (years)	10.0	14.5	11.9	12.1
% with poor cognitive outcomes	50%	70%	76%	66%

* Poor outcome included individuals functioning in the range of delayed cognitive development (Bayley Cognitive Score less than Standard Score of 70) or in the range consistent with an intellectual disability (ie, Bayley Cognitive Scale or FSIQ scores from the WASI/WPPSI-3 < 70).

Table II

Demographic data by diagnosis

		CPSD/OTCD (n=38)	ASD (n=37)	ALD (n=28)	p-value
Sex	Female n (%)	4 (10)	22 (59)	15 (54)	<0.001
Race	Black	4 (11.4)	2 (6.1)	1 (4.8)	
	White	29 (82.9)	21 (63.6)	23 (82.1)	0.024
	Asian	1 (2.9)	10 (30.3)	4 (14.3)	
Ethnicity	Hispanic	9 (24.3)	7 (20.0)	2 (7.4)	
	Non-Hispanic	28 (75.7)	28 (80.0)	25 (92.6)	0.221
Parent education	<High School (<12y)	7 (25.0)	2 (8.0)	3 (15.8)	
	High School (12 y)	5 (17.9)	7 (28.0)	3 (15.8)	
	Some College (13–15y)	6 (21.4)	8 (32.0)	3 (15.8)	0.544
	College Grad (16 y)	5 (17.9)	2 (8.0)	5 (26.3)	
	Post Graduate (16+ y)	5 (17.9)	6 (24.0)	5 (26.3)	
Family Income (\$) Per Dependent	<7500	6 (26.1)	4 (23.5)	5 (29.4)	
	7500–12500	4 (17.4)	1 (5.9)	4 (23.5)	
	12500–17500	6 (26.1)	2 (11.8)	2 (11.8)	0.715
	17500–23333	3 (13.0)	3 (17.6)	2 (11.8)	
	23333–50000	4 (17.4)	7 (41.2)	4 (23.5)	

Table III

Initial hyperammonemia episode by diagnosis

		CPSD/OTCD(n=38)	ASD(n=37)	ALD(n=28)	p-value
Age (days) at first admission	2	21 (55.2)	10 (27.0)	8 (28.5)	
	3–7	11 (28.9)	25 (67.6)	16 (57.1)	0.013
	>7	6 (15.7)	2 (5.4)	4 (14.3)	
Length of stay of first admission (days)	<1 week	0 (0.0)	2 (5.6)	3 (10.7)	
	1–<2 weeks	7 (18.9)	7 (19.4)	12 (42.9)	0.057
	2–<4 weeks	13 (35.1)	15 (41.7)	6 (21.4)	
	4 weeks	17 (46.0)	12 (33.3)	7 (25.0)	
	200–399	2 (6.7)	4 (21.0)	7 (43.7)	
Peak ammonia level (μmol/L)	400–599	5 (16.7)	3 (15.8)	1 (6.2)	
	600–799	5 (16.7)	4 (21.0)	2 (12.5)	0.094
	800–999	1 (3.3)	2 (10.5)	2 (12.5)	
	1000	17 (56.7)	6 (31.6)	4 (25.0)	
	Mean (95% CI)	963 (746, 1243)	589.2 (432, 803)	573.5 (402, 818)	0.019
	<41	23 (67.6)	21 (60.0)	8 (29.6)	
AST Level (U/L)	41–54	6 (17.6)	9 (25.7)	4 (14.8)	
	55–69	2 (5.9)	2 (5.7)	1 (3.7)	0.005
	70–99	3 (8.8)	2 (5.7)	7 (25.9)	
	100–149	0 (0.0)	0 (0.0)	3 (11.1)	
	150	0 (0.0)	1 (2.9)	4 (14.8)	
ALT Level (U/L)	<57	29 (85.3)	25 (73.5)	8 (29.6)	
	57–69	0 (0.0)	2 (5.9)	2 (10.0)	
	70–99	3 (8.8)	4 (11.8)	3 (11.1)	<0.001
	100–149	2 (5.9)	2 (5.9)	6 (22.2)	
	150–299	0 (0.0)	1 (2.9)	4 (14.8)	
	300	1 (0.0)	0 (0.0)	4 (14.8)	

Table IV

Age at liver transplant by diagnosis

Age at liver transplant (months)	No liver transplant	CPSPD/OTCD (n=38)	ASD (n=37)	ALD (n=28)	p value
		13 (34.2)	27 (73.0)	16 (64.3)	
		7 (18.4)	1 (2.7)	0 (0.0)	
	<6				
	6–<12	11 (28.9)	2 (5.4)	1 (3.6)	
	12–<24	3 (7.9)	6 (16.2)	2 (7.1)	<0.001
	24–<48	2 (5.3)	0 (0.0)	1 (3.6)	
	48	2 (5.3)	1 (2.7)	6 (21.4)	